MARYLAND BIOLOGICAL STREAM SURVEY 2000-2004

Volume VI

Laboratory, Field, and Analytical Methods

MARYLAND DEPARTMENT OF NATURAL RESOURCES

CHESAPEAKE BAY AND WATERSHED PROGRAMS
MONITORING AND NON-TIDAL ASSESSMENT
CBWP-MANTA-EA-05-3

Robert L. Ehrlich, Jr.
Governor

Michael S. Steele
Lt. Governor

A message to Maryland's citizens

The Maryland Department of Natural Resources (DNR) seeks to preserve, protect and enhance the living resources of the state. Working in partnership with the citizens of Maryland, this worthwhile goal will become a reality. This publication provides information that will increase your understanding of how DNR strives to reach that goal through its many diverse programs.

C. Ronald Franks
Secretary

MARYLAND
DEPARTMENT OF
NATURAL RESOURCES

Tawes State Office Building
580 Taylor Avenue
Annapolis, Maryland 21401

Toll free in Maryland: 1-(877)- 620-8DNR ext. 8610
Out of state call: 410-260-8610
TTY via Maryland Relay: 711 (within MD)
800-735-2258 (out of state)
www.dnr.maryland.gov

Publication # DNR-12-0305-0108

Published July 2005

PRINTED ON RECYCLED PAPER

Maryland Biological Stream Survey 2000-2004

Volume 6: Laboratory, Field, and Analytical Methods

Prepared for

Maryland Department of Natural Resources
Monitoring and Non-tidal Assessment Division
Tawes State Office Building
580 Taylor Avenue
Annapolis, Maryland 21401

Prepared by

Nancy Roth
Jon Vølstad
Lori Erb
Ed Weber
Versar, Inc.
9200 Rumsey Road
Columbia, MD 21045

and

Paul Kazyak
Scott Stranko
Dan Boward
Maryland Department of Natural Resources
Tawes State Office Building
580 Taylor Avenue
Annapolis, Maryland 21401

July 2005

PUB # DNR 12-0305-0108
EA-05-3

FOREWORD

This report, *Maryland Biological Stream Survey 2000-2004 Volume 6: Laboratory, Field, and Analytical Methods*, was prepared by Versar, Inc., for the Maryland Department of Natural Resources' Monitoring and Non-Tidal Assessment Division. It was supported by Maryland's Power Plant Research Program (Contract No. K00B0200109 to Versar, Inc.).

ACKNOWLEDGMENTS

All aspects of the Maryland Biological Stream Survey have been a cooperative effort among Maryland DNR, several academic institutions, and consulting firms, as listed in the acknowledgements sections of each volume. For this volume, the authors would like to acknowledge the following individuals who contributed data analysis, graphical support, editing, or other talents:

Versar	**DNR**
Beth Franks	Ron Klauda
Gail Lucas	
Sherian George	

ABSTRACT

The Maryland Biological Stream Survey (MBSS) is a statewide probability-based sample survey for assessing the status and trends in the water chemistry, physical habitat, and biological condition of wadeable, non-tidal streams in Maryland. A lattice sampling design was used to select watersheds randomly in time and space, while sites along the network of streams within each watershed were selected by stratified or simple random sampling. New management objectives resulted in a change in design from the 1995-1997 MBSS, with the 2000-2004 MBSS focusing on the assessment of smaller Maryland 8-digit watersheds, instead of 6-digit basins. The state was stratified into five regions, and $1/5^{th}$ of the watersheds was selected from each region annually to facilitate cost-effective use of field crews. Field data were collected from a representative sample of at least ten 75-m stream segments from each watershed using a combination of quantitative and qualitative methods. Biological indicators for fish and benthic macroinvertebrate assemblages developed by the MBSS provide the basis for the State's biocriteria framework and 303d listing of impaired waters under the Clean Water Act. This Volume describes in detail the field, laboratory, and analytical methods for the 2000-2004 MBSS, including estimators of stream condition at multiple spatial scales, from individual watersheds to the entire state. Methods for integrating results from additional sample programs with MBSS are provided, including the combination of MBSS and County estimates of stream condition.

TABLE OF CONTENTS

Page

TABLE OF CONTENTS (CONTINUED)

Page

LIST OF TABLES

Table No. **Page**

LIST OF FIGURES

Figure No. **Page**

6.1 BACKGROUND

This chapter presents the study design and procedures used to implement Round Two of the Maryland Biological Stream Survey (MBSS or the Survey). Details of the study design and sample frame are included below, along with a summary of landowner permission results and the number of sites sampled in watersheds selected for sampling in 2000-2004. This background material is followed by a summary of field and laboratory methods for each component: water chemistry, benthic macroinvertebrates, fish, amphibians and reptiles, vegetation, and physical habitat. Quality assurance (QA) activities are also described. For further details on Round Two methods, see the MBSS Sampling Manual (Kazyak 2000).

In principle, the survey methods used in Round Two of the MBSS (2000-2004) were comparable to those of Round One (1995-1997). However, some changes in survey design and field data collections were made to accommodate new objectives of MBSS and to improve the quality and/or usefulness of the data generated. The changes in field sampling methods included (1) modifications to the physical habitat assessment and characterization, (2) the addition of new chemical analytes (total nitrogen, nitrite, ammonia, orthophosphate, total phosphorous, chloride, and turbidity), (3) collection of continuous in-stream temperature readings at all randomly-selected sample sites throughout the summer, and (4) characterization of invasive terrestrial plant abundance. The survey design was modified in several ways to support reliable estimates of stream condition within 8-digit watershed boundaries. The reach file (sampling frame) used to select 8-digit watersheds and sites within watersheds was the 1:100,000-scale map developed by USGS; this was a change from the 1:250,000-scale map used in Round One. Another change to the sample frame was the expansion of the MBSS to include fourth-order, non-tidal streams.

6.2 SAMPLE SURVEY DESIGN

The second round of the MBSS was conducted over five years and started in the year 2000. The Round Two Survey was designed to provide an assessment of stream condition in each of the Maryland 8-digit watersheds that contain non-tidal streams. It also facilitates the assessment of average stream condition over the five-year period for (1) the entire state, (2) the 17 major (Maryland 6-digit) drainage basins, and (3) other areas of interest such as counties and regions. The design was subject to the following level-of-effort constraints: (1) that a maximum of 300 sites be sampled per year, with approximately 210 allocated to the core random design, and (2) that the maximum sampling interval be 5 years.

6.2.1 Sample Frame

The sample frame for the 2000-2004 MBSS was based on the 1:100,000-scale stream network, a map scale consistent with that used by EPA and other states. The Maryland 8-digit watersheds in this network defined primary sampling units (PSUs). Maryland defines 8-digit watersheds at a scale finer than the USGS 8-digit Hydrologic Cataloging Units (HUCs). In most, but not all cases, these state-defined units are true topographic watersheds (Omernik and Bailey 1997). Maryland 8-digit watersheds (average area 194 km^2) are subunits of USGS 8-digit HUCs (average area in Maryland 1295 km^2). The frame was constructed by overlaying the 138 Maryland 8-digit watershed boundaries (Figure 6-1) on a map of all stream reaches in the study area as digitized on a U.S. Geological Survey 1:100,000-scale map. It included all non-tidal stream reaches of fourth-order and smaller, excluding impoundments that are non-wadeable or that substantially alter the riverine nature of the reach (see Kazyak 1994). Fourth-order streams were included to expand statewide coverage and ensure that all the streams classified as third-order by the 1:250,000 map (and sampled in the 1995-1997 MBSS) were also covered in the 2000-2004 MBSS. Four 8-digit watersheds (Atlantic Ocean, plus the Upper, Middle, and Lower Chesapeake Bay) were excluded from the sample frame because they describe marine/estuarine waters and do not contain non-tidal streams. Of the 134 watersheds included in the frame, 79 contained less than 100 non-tidal stream miles each; these were combined into 29 “super-watersheds” with between 2 and 7 constituent 8-digit watersheds each. When combined with the 55 remaining “stand alone” watersheds, a total of 84 watersheds of concern were identified as discrete sampling units for Round Two (Table 6-1). It should be noted that the PSUs visited for the 2000-2004 MBSS often are not true watersheds, but rather sampling units of generally similar size that are watershed-based to the extent practicable.

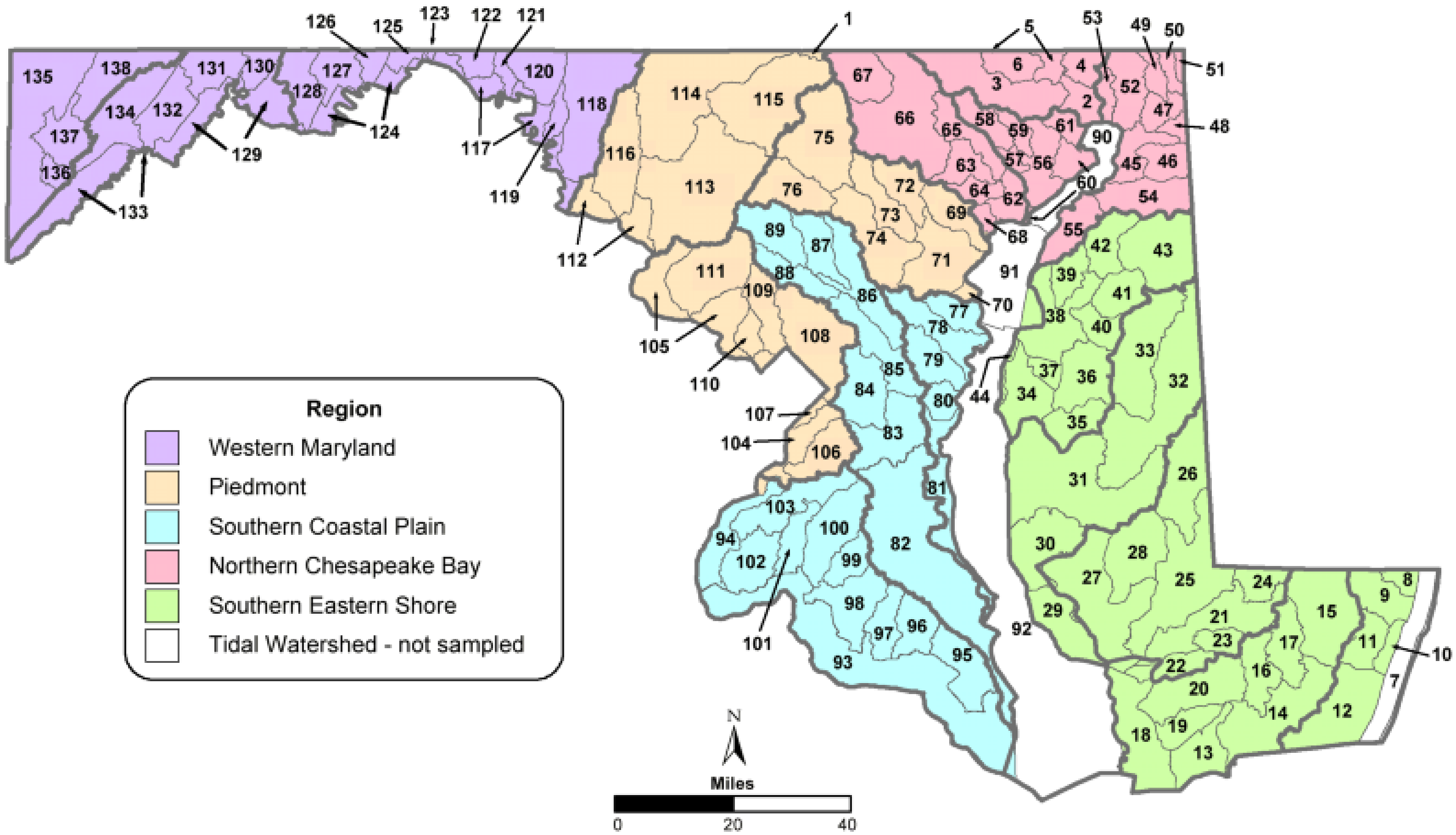

Figure 6-1. Maryland 8-digit watersheds by region

Table 6-1. Maryland individual and combined watersheds (primary sampling units or PSUs) sampled in the 2000-2004 MBSS. * indicates watershed selected that year for repeated sampling

Basin	Watershed	Watershed Number	2000	2001	2002	2003	2004	Extra Sites
Youghiogheny	Youghiogheny River	135		X				6
	Little Youghiogheny/Deep Creek Lake	136/137					X	
	Casselman River	138	X					
North Branch Potomac	Potomac River Lower North Branch	129				X		5
	Evitts Creek	130					X	
	Wills Creek	131					X	
	Georges Creek	132				X		
	Potomac River Upper North Branch	133		X				
	Savage River	134			X			4
Upper Potomac	Antietam Creek	118				X		4
	Potomac WA Co/Marsh Run/Tonoloway/Little Tonoloway	117/119/123/125	X		*			3
	Conococheague	120			X			
	Little Conococheague/Licking Creek	121/122					X	
	Potomac Allegany County/Sideling Hill Creek	124/126		X				
	Fifteen Mile Creek	127	X					
	Town Creek	128	*		X			
Middle Potomac	Potomac River FR Co	112					X	
	Lower Monocacy River	113				X		11
	Upper Monocacy River	114	X					8
	Conewago Creek/Double Pipe Creek	1/115			X			7
	Catoctin Creek	116				X		4
Potomac Wash Metro	Potomac River Montgomery County	105			X			5
	Piscataway Creek	106		X				
	Potomac Upper Tidal/Oxon Creek	104/107		X				
	Anacostia River	108					X	5
	Rock Creek/Cabin John Creek	109/110				X		
	Seneca Creek	111		X				5
Patapsco	Back River	69			X			
	Bodkin Creek/Baltimore Harbor	70/71		X			*	
	Jones Falls	72			X			
	Gwynns Falls	73					X	
	Patapsco River Lower North Branch	74	X					4
	Liberty Reservoir	75	X			*		5
	South Branch Patapsco	76	X					

Table 6-1. (Continued)

Basin	Watershed	Watershed Number	2000	2001	2002	2003	2004	Extra Sites
Patuxent	Little Patuxent River	86	X					3
	Middle Patuxent River	87			X			
	Rocky Gorge Dam	88			X			
	Brighton Dam	89	X					
	Patuxent River Lower	82					X	8
	Patuxent River Middle	83		X				3
	Western Branch	84		X				
	Patuxent River Upper	85					X	
Lower Potomac	Breton/St. Clements Bays	96/97			X			
	Potomac Lower Tidal/Potomac Middle Tidal	93/94			*		X	
	St. Mary's River	95	*			X		
	Wicomico River	98					X	
	Gilbert Swamp	99		X				
	Zekiah Swamp	100		X				3
	Port Tobacco River	101				X		
	Nanjemoy Creek	102	X					
	Mattawoman Creek	103	X					
West Chesapeake	Magothy River/Severn River	77/78				X		
	South River/West River	79/80			X			
	West Chesapeake Bay	81				X		
Gunpowder	Gunpowder River/Lower Gunpowder Falls/Bird River/ Middle River-Browns	62/63/64/68			X			
	Little Gunpowder Falls	65		*		X		
	Loch Raven Reservoir	66			X			7
	Prettyboy Reservoir	67	X					
Susquehanna	Lower Susquehanna/Octoraro Creek/Conowingo Dam Susquehanna	2/4/5					X	
	Deer Creek	3		X			*	4
	Broad Creek	6				X		
Bush	Aberdeen Proving Ground/Swan Creek	60/61	X					
	Lower Winters Run/Atkisson Reservoir	57/58					X	
	Bush River/Bynum Run	56/59					X	
Elk	Northeast River/Furnace Bay	52/53		X				
	Lower Elk River/Bohemia River/Upper Elk River/Back Creek/Little Elk Creek/Big Elk Creek/Christina River	45/46/47/48/49/50/51				X		
	Sassafras River/Stillpond-Fairlee	54/55		X				

Table 6-1. (Continued)

Basin	Watershed	Watershed Number	2000	2001	2002	2003	2004	Extra Sites
Chester	Eastern Bay/Kent Narrows/Lower Chester River/ Langford Creek/Kent Island Bay	34/37/38/39/44			X			
	Miles River/Wye River	35/36				X		
	Corsica River/Southeast Creek	40/41	X					
	Middle Chester River	42			X	*		
	Upper Chester River	43					X	
Choptank	Honga River/Little Choptank/Lower Choptank	29/30/31				X		
	Upper Choptank	32	X					
	Tuckahoe Creek	33				X		
Nanticoke/Wicomico	Lower Wicomico/Monie Bay/Wicomico Creek/Wicomico River Head	21/22/23/24	X					
	Nanticoke River	25		*	X			
	Marshyhope Creek	26					X	
	Fishing Bay/Transquaking River	27/28					X	
Pocomoke	Pocomoke Sound/Tangier Sound/Big Annemessex/Manokin River	13/18/19/20				X		
	Lower Pocomoke River	14			X			
	Upper Pocomoke River	15		X				3
	Dividing Creek/Nassawango Creek	16/17		X				
Ocean Coastal	Assawoman/Isle of Wight/Sinepuxent/Newport/Chincoteague Bays	8/9/10/11/12		X				
Other	Upper Chesapeake Bay/Middle Chesapeake Bay/Lower Chesapeake Bay/Atlantic Ocean	90/91/92/7						
Total			18	19	19	19	19	107

The Strahler convention (Strahler 1957) was used for identifying stream reaches in each 8-digit watershed by order. First order reaches, for example, are the most upstream reaches in the branching stream system. The designation of stream order for a particular reach depends on the scale and accuracy of the map.

6.2.2 Sample Selection

The second round of MBSS was restricted to a maximum of 300 sampling sites per year (210 within the core survey; the remainder of sites were reserved for targeted, non-random sampling to support a variety of other activities). Hence, it was not practical to stratify the network of streams in Maryland by 8-digit watersheds and sample them annually (i.e., only 2 sites could be sampled in each of the 134 watersheds each year under that design, resulting in unreliable estimates at the 8-digit watershed scale). In addition, the costs of traveling to sample each watershed each year would be high. As an alternative to stratifying by watershed, the MBSS designated the 84 watershed units of concern (both 55 single watershed units and 29 super-watersheds) as primary sampling units (PSUs). A subset of the 84 PSUs was selected randomly each year, with restrictions to ensure that all 8-digit watersheds were sampled once during the five-year sampling period. Using this approach, a representative subset of watersheds was studied each year, covering all the 84 watersheds of concern over a five-year period. The sampling within a subset of watersheds reduced travel time, thus increasing the number of sites that could be sampled for a fixed cost as compared to stratified random sampling.

6.2.2.1 Lattice Sampling of Watersheds (PSUs)

Lattice sampling was used to schedule the sampling of all 84 watersheds (PSUs) over a five-year period (see Cochran 1977; Jessen 1978). A sampling frame for selecting watersheds across time was formed by arranging the PSUs into a lattice with 84 rows and one column for each year (Table 6-1).

The 84 PSUs were stratified into five regions (strata) to ensure that their sampling was spread out geographically during each sample year (Figure 6-2). These five regions include whole major (Maryland 6-digit) drainage basins and divide the State into approximately equal parts. This stratification by region was done to spread out the sampling in space and thereby increase precision in statewide estimates; the geographic strata were not considered important reporting units.

A first-stage random sample of PSUs was drawn from each region in each year, with restrictions to ensure that all 84 watersheds (PSUs) of concern were sampled at least once during the five-year sampling period. The lattice sampling supports an estimate of average statewide condition over the five-year period. This strategy is similar to the lattice design used in the 1994 Demonstration Study (Vølstad et al. 1996) and the 1995-1997 MBSS Round One design (Roth et al. 1999); it takes into account the restrictions in annual sampling effort. About one-fifth of the watersheds in each of the five regions were randomly selected (without replacement) each year. In addition, two randomly selected watersheds in each region were being sampled twice during the five-year MBSS (in randomly selected years). The representative sampling over time, augmented by repeated sampling of watersheds, ensures that all PSUs and pairs of PSU combinations have a known probability (greater than zero) of being selected. This probability-based sampling facilitates the estimation of statewide average condition over the five-year study period with quantifiable precision based on the Horvitz-Thompson estimator (Horvitz and Thompson 1952; Thompson 1992). It also allows estimation of statewide conditions for each year of the MBSS.

6.2.2.2 Stratified Random Sampling within PSUs

Within each PSU, the elementary sampling units from which field data were collected (i.e., the 75-m stream segments or sites) were selected using either stratified random sampling with proportional allocation, or simple random sampling (Cochran 1977). This allocation ensures that all sites in a PSU stream network have the same probability of being selected. The target sample size in each PSU had a minimum of 10 sites for the spring benthic sampling. Because of imperfections in the sample frame, a list of random replacement sites was provided for each PSU.

When the Round Two design was proposed, the target minimum of 10 sites per PSU was determined by analyzing the expected variability in IBI mean scores and percentage stream mile estimates as a function of varying sample size. Analysis (as presented in Southerland et al. 2000) indicated that fewer than 10 sites per PSU would not yield sufficient precision in stream mile estimates. Working with DNR, the survey designers determined that 10 sites per watershed would yield an acceptable level of precision while remaining within other design constraints (i.e., the annual level of effort available for sampling and the maximum sampling interval of five years for the statewide survey).

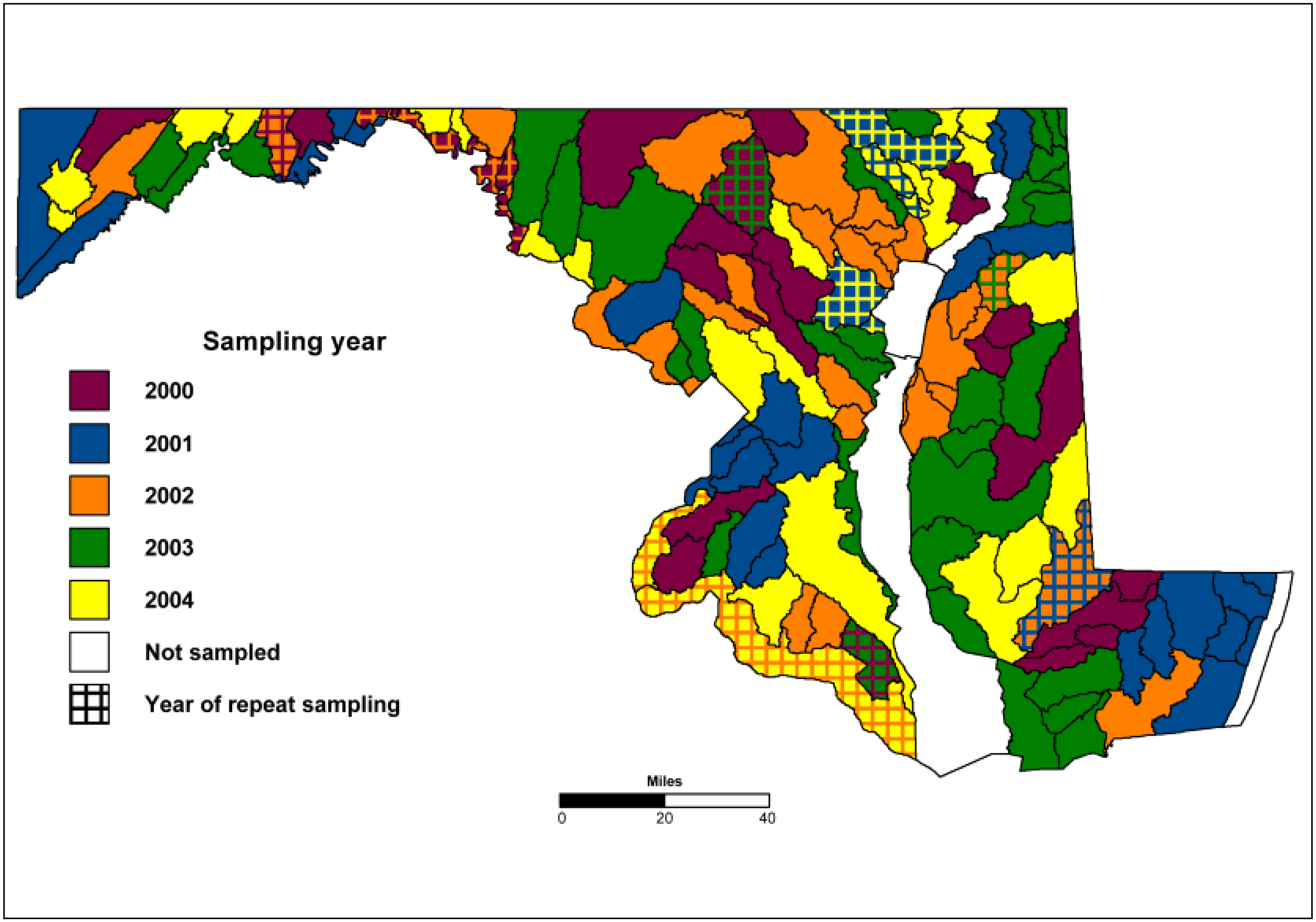

Figure 6-2. MBSS 2000-2004 Primary Sampling Units (PSU)s and sampling schedule

When feasible, the streams in each of the 55 PSUs consisting of a single 8-digit watershed were grouped into two strata based on stream order. One stratum included all the first- and second-order streams, while the other included all the third- and fourth-order streams. The number of sites in each of the two strata was allocated proportional to their stream length, resulting in equal sampling density for the two strata. In watersheds where the proportion of stream miles in one stratum (e.g., third- and fourth-order streams) was below 10%, the stringent proportional allocation could not be achieved because it would result in allocation of less than one sample site to this stratum. Samples were not forced into strata that contained a minimal portion of stream miles, because this would eliminate the simplicity of equal probability sampling. Instead, the strata for such PSUs were collapsed, and a simple random sample of sites from all streams was selected.

A different stratification was used for the 29 PSUs consisting of more than one 8-digit watershed (i.e., the super-watersheds). For these PSUs, each constituent 8-digit watershed was designated a stratum, and the strata receive equal sampling fractions (i.e., proportional to stream miles in each 8-digit watershed). This stratification of super-watersheds was done to ensure that the non-tidal streams in each individual 8-digit watershed were sampled. While this approach may increase precision of stratified estimates for the super-watershed, the precision in estimates for individual 8-digit watersheds will generally be low because of low sample sizes. The limited sample sizes allocated to each PSU did not allow further stratification of the super-watersheds by stream order.

When one or more of the initial sample of stream segments in a PSU could not be sampled (e.g., dry stream or no permission to access), the stratification of the PSU was abandoned, and the replacement sites were selected from a list of simple random sites. This adjustment was made because the fraction of unsampleable sites cannot be adequately quantified for individual strata with low sample sizes.

6.2.2.3 Allocation of Additional Sites to Large Watersheds

Additional sites were allocated to 22 watersheds with more than 100 non-tidal stream miles. Increased sample sizes in these watersheds reduced the variance of key estimates and improved statewide estimates (by more closely approximating statewide allocation proportional to stream miles). Over the five-year Survey, a total of 106 additional sites were allocated proportional to stream miles within these large watersheds (Table 6-2).

Table 6-2. List of MBSS Round Two Primary Sampling Units with greater than 100 non-tidal stream miles, scheduled for additional sample sites

Primary Sampling Unit	Number of Stream Miles	Number of Additional Sites
Lower Monocacy River	388.39	11
Upper Monocacy River	284.38	8
Patuxent River Lower	280.90	8
Loch Raven Reservoir	237.10	7
Conewago Creek/Double Pipe Creek	231.16	7
Youghiogheny River	222.56	6
Liberty Reservoir	184.08	5
Seneca Creek	178.85	5
Potomac River Lower North Branch	165.45	5
Potomac River Montgomery County	160.68	5
Anacostia River	159.34	5
Antietam Creek	146.34	4
Deer Creek	142.62	4
Patapsco River Lower North Branch	129.50	4
Catoctin Creek	128.95	4
Savage River	127.13	4
Upper Choptank	127.02	4
Little Patuxent River	122.48	3
Zekiah Swamp	120.75	3
Potomac Washington County/Marsh Run/Tonoloway/Little Tonoloway	118.43	3
Patuxent River Middle	111.19	3
Upper Pocomoke River	109.65	3

6.2.3 Site Selection

- Sample Frame Construction. The stream order of each reach was attributed on the 1:100,000-scale USGS Digital Line Graph (DLG) maps. When necessary, 1:24,000-scale USGS topographic maps were used as references to identify flow patterns or to see more detail. Maps from Pennsylvania and Delaware were also used to identify the stream order of water bodies originating outside of Maryland.

- Random Site Picks. Once the sample frame was developed for a PSU, sites were randomly assigned according to the stratified design described above using a FORTRAN-based program. If the proportion of stream miles in the smallest strata (either stream-order-based in single watershed PSUs or watershed-based in the super-watersheds) was greater than or equal to 10%, sites were allocated proportionally among strata; if it was less than 10%, the strata were collapsed and sites allocated by simple random sampling. After the target number of sites was selected (10 to 21 sites depending on PSU size), a simple random selection of “extra sites” to a total of 50 was chosen in each PSU using the GIS. This was done to ensure that a sufficient number of sites remained available for sampling after permission denials and unsampleable sites were removed from consideration.

- Each sample point chosen on the GIS was designated as the midpoint of the 75-m sampling segment in the field. Sites selected less than 75 meters from another randomly-selected site (both upstream and downstream) were eliminated. Sites that could possibly cross stream network nodes were not eliminated from the program; it was assumed that these sites could be adjusted in the field by moving the starting point away from the node, but staying within the designated stream order.

Each site was then attributed with the following information:

- stream order
- county
- basin
- physiographic region
- northing, easting
- latitude and longitude (both in decimal degrees and in degrees, minutes, seconds)
- watershed name and MD 8-digit watershed code.

6.2.4 Permissions from Landowners

- <u>Extra Permissions</u>. Permission was solicited to sample from landowners at twice the number of sites allocated to each PSU by the design (usually 20 sites, but from 26 to 42 in the larger watersheds). While the allocated number of sites (usually 10) were selected from the appropriate strata (see above), the “extra sites” were chosen to fill out the list, regardless of stream order. At the completion of site selection for each county, sites were sent to DNR for generation of 1:24,000-scale topographic maps and communication of sites to local governments planning stream monitoring.

- <u>Landowner Identification</u>. Each site was plotted on county tax maps using the Maryland Office of Planning Maryland Property View System obtained from DNR. From this, property owners could be identified, both for the site containing the sampling site and for any areas required to access the stream. Phone numbers were obtained from the internet using a white pages directory (http://www.switchboard.com).

- <u>Landowner Contact</u>. Letters were prepared requesting permission to access the property, including a written form and telephone contact information through which the landowner could respond. The letter also provided a MBSS brochure and telephone number to call for more information. If no response was received from the mailings and the phone number was listed, the property owner was called and permission to access the site was requested. If the owner gave permission, the caller requested additional information about the site, such as whether the stream was often dry or hard to access. The caller also recorded whether the crew needed to make a pre-visit call to the landowner or whether the owner had to be available to open gates or walk the crew through the property. All property owner information was entered and maintained in a Microsoft Access database.

- <u>Field Crew Information</u>. Permission packets were then prepared for the field crews. Packets contained a printout of the property owner information for each site and a tax map showing possible access routes. The callers attempted to obtain permissions for the target sites in the proportions that stream orders occur in each PSU. In addition, permissions were obtained for extra sites (up to 50% more than the targeted number) to account for non-sampleable sites. These extra sites represent a simple random sample and may or may not have been of the same stream order as the originally selected sites (for example, if a third- to fourth-order site was unsampleable, the replacement site was the next on the simple random list, regardless of stream order).

6.3 ANALYTICAL METHODS

6.3.1 Standard Estimators for the MBSS Sampling Program

6.3.1.1 Overview

Estimates for the MBSS sampling program were calculated using standard estimators under simple random sampling, cluster sampling with primary units of unequal size, or stratified random sampling designs (Cochran 1977) as appropriate. The estimators used depended on the spatial scale at which the variable was estimated. Within a PSU, simple random sampling estimators were used because all samples within a PSU had equal probability of being selected. Within larger spatial units (MDE 6-digit basin, county, geographic region, or the entire state), estimates were based on cluster sampling for yearly estimates and stratified sampling for whole-round estimates. This is because a randomly selected portion of all PSUs were sampled each year during the round, necessitating the use of cluster sampling for yearly estimates. However, all PSUs were sampled by the end of the round, thereby reducing the estimator for a cluster sample to a stratified random sample estimator through the finite population correction for stage one (*cf.* Cochran 1977).

The cluster- or stratified-sampling estimators, rather than the Horvitz-Thompson estimator (Horvitz and Thompson 1952), were used to calculate parameters of interest for larger spatial units for simplicity. Although a small fraction of PSUs was resampled as part of the lattice sampling described above, we did not include an estimate of temporal variation for statewide estimates of stream condition for two reasons. First, preliminary yearly estimates for PSUs with replicate samples over time indicated that temporal variation within PSUs was very small compared to spatial variation for most variables of interest. Second, treating the set of all samples within a PSU as a simple random sample allowed for calculation of a single whole-round estimate for a variable of interest. For each PSU with replicate samples over time, the variance in single parameter estimates includes temporal as well as spatial variability. Presenting a single estimate for each PSU rather than several estimates may be more useful for regulatory agencies (i.e., MDE). To be consistent, these within-PSU estimates were treated as strata or clusters to estimate parameters over larger spatial units. Although we did not take advantage of the resampling design fully, it will likely be useful for time-series analysis as the MBSS program collects additional data in future rounds of sampling.

We list the estimators used in the MBSS program by sampling type below, and summarize the symbols used in estimators in Table 6-3.

Table 6-3. Symbols that refer to the population of streams and the sample of sites

Population	Sample	6.3.1.1.1 Defined as
N_r	n_r	Number of watersheds (PSUs) in a basin, region, or the state (*r*)
M_{ir}	m_{ir}	Number of 75-m sites in PSU *i* within region or basin *r*, or the entire state
Y_{jir}	y_{jir}	Variable of interest associated with site *j*, j=1,2,...,m_{ri}

6.3.1.2 Estimation of Means and Totals within Watersheds (PSUs)

Estimates of means, proportions, and totals within a PSU were calculated using estimators for simple random sampling. For a variable of interest, the sample mean was estimated as

$$\bar{y}_{ri} = \frac{1}{m_{ri}} \sum_{j=1}^{m_{ir}} y_{rij} \ .$$

The variance of $\bar{y}_{ri}$ was calculated as

$$\widehat{\mathrm{var}}(\bar{y}_{ri}) = \frac{s^2_{y_{ri}}}{m_{ri}} ,$$

where

$$s^2_{y_{ri}} = \frac{\sum_{j=1}^{m_{ri}} \left(y_{rij} - \bar{y}_{ri} \right)^2}{m_{ri} - 1} .$$

The total for a variable of interest, Y_i , was estimated by expanding the mean to all units

$$\hat{Y}_{ri} = M_{ri} \bar{y}_{ri} ,$$

with variance estimated as

$$\hat{V}(\hat{Y}_{ri}) = M_{ri}{}^2 \, \widehat{\mathrm{var}}(\bar{y}_{ri}) \ .$$

6.3.1.3 Estimation of Yearly Means and Totals for State, Region, and Basin

Yearly estimates at the state, region, or basin level were calculated using estimators for cluster sampling with primary units of unequal size. The mean of a variable of interest was estimated as

$$\bar{y}_r = \frac{\sum_{i=1}^{n_r} M_{ri}\bar{y}_{ri}}{\sum_{i=1}^{n_r} M_{ri}}$$

with variance

$$\widehat{\mathrm{var}}(\bar{y}_r) = \frac{1-(n_r/N_r)}{\bar{M}_r^2 n_r(n_r-1)}\sum_{i=1}^{n_r} M_{ri}^2(\bar{y}_{ri}-\bar{y}_r)^2 + \frac{1}{n_r N_r \bar{M}_r^2}\left[\sum_{i=1}^{n_r} M_{ri}^2 \frac{s_{2i}^2}{m_{ri}}\right],$$

where

$$s_{2i}^2 = \frac{1}{m_{ri}-1}\sum_{j=1}^{m_{ri}}(y_{rij}-\bar{y}_{ri})^2 .$$

The total for a variable of interest was estimated by

$$\hat{Y}_r = \frac{N_r}{n_r}\sum_{i=1}^{n_r} M_{ri}\bar{y}_{ri}$$

with variance

$$\hat{V}(\hat{Y}_r) = N_r^2\,\widehat{\mathrm{var}}(\bar{y}_r) .$$

6.3.1.4 Estimation of Means and Totals for State, Region, and Basin for the five-year period

Stratified estimators were used to estimate means, proportions, and totals for statewide, regional, and basinwide estimates, with PSUs treated as strata because of complete sampling coverage (i.e., all PSUs were sampled during the round). An estimator for the mean of the variable of interest y is

$$\bar{y}_r = \sum_{i=1}^{N_r} w_{ri}\bar{y}_{ri} ,$$

where

$$w_r = \frac{M_r}{\sum_{i=1}^{N_r} M_{ri}}$$

The variance of the stratified mean for y in area r was estimated as

$$Var(\bar{y}_r) = \sum_{n_r=1}^{N_r} w_{ri}^2 \frac{s_{ri}^2}{m_{ri}} ,$$

where

$$s_{ri}^2 = \frac{(y_{ri}-\bar{y})^2}{(m_{ri}-1)}$$

An estimate for the total of a variable of interest in a watershed i was obtained by extrapolating the mean to all stream miles

$$\bar{Y}_r = M_r\bar{y}_r$$

Although a few PSUs had streams that overlapped regions slightly, each PSU was assigned entirely to the region where most of its stream miles occurred for these estimates.

6.3.1.5 County Estimates

County estimates were calculated by stratified random sample estimators, similar to those used for regions and basins. However, PSUs that crossed county borders were post-stratified. Only the portion of stream mileage in each PSU (M_{ir}) that occurred within the county was included in these calculations. Because estimates for fish populations were pooled over 75-m sites within PSUs, and sample sizes were relatively small, we assumed that fish density was equal throughout each PSU so that fewer estimates failed. That is, the mean density calculated for a PSU that crosses a county border was applied to the portion of the PSU within the county (post-stratum), and weighting was based on the stream mileage within the post-stratum when deriving stratified county estimates.

6.3.1.6 Estimates of Proportions

The estimators for means were also used to estimate proportions of stream miles in a specific class by introducing an indicator variable that took the value 1 when the variable y met the condition (e.g., pH < 6), and zero otherwise. The mean of this indicator using the estimators above was an estimate of the proportion of stream miles within the specific class (e.g., proportion of stream miles with pH < 6). When estimating proportions within PSUs, the MBSS samples were treated as repeated independent samples of binary observations (e.g., 1 if pH < 6, and 0 otherwise) because the samples had equal inclusion probabilities. In some cases, an exact confidence interval for an estimated proportion (p) was obtained from the binomial distribution (Collett 1999, pp. 23-24), with lower and upper confidence bounds

$$p_L = y[y + (n - y + 1)F_{2(n-y+1),2y}(\alpha/2)]^{-1}$$

$$p_U = (y+1)[y+1+(n-y)F_{2(y+1),2(n-y)}(\alpha/2)]^{-1}$$

respectively, where $F_{v1,v2}(\alpha/2)$ was the upper $(100\alpha/2)$% point in the F-distribution with v_1 and v_2 degrees of freedom, and y was the observed number of successes (e.g., number of sites with IBI < 3) out of the n observations in a watershed.

6.3.1.7 Estimators for Combining MBSS with Additional Probability-based Sampling Programs

When additional MBSS compatible data for a spatial area were available from a probability-based sampling program, the results for that area were combined by using a composite estimator (Vølstad et al. 2002). Assume that MBSS and a County program provide simultaneous estimates of the mean IBI for a watershed, and that the primary objective is to obtain a unified estimate of stream condition with less variance than the individual estimates. If the two programs cover the same network of streams, a unified mean IBI for the watershed is estimated by combining the individual survey means $\bar{y}_1$ and $\bar{y}_2$, using the composite estimator (Rao 2003)

$$\bar{y}_C = \phi\bar{y}_1 + (1-\phi)\bar{y}_2,$$

with variance

$$\text{var}(\bar{y}_C) = \phi^2\,\text{var}(\bar{y}_1) + (1-\phi)^2\,\text{var}(\bar{y}_2).$$

If $\bar{y}_1$ and $\bar{y}_2$ are approximately unbiased for the population mean IBI, then $\bar{y}_C$ will also be unbiased. The variance of $\bar{y}_C$ is minimized by using the weight

$$\phi = \frac{\text{var}(\bar{y}_2)}{\text{var}(\bar{y}_1) + \text{var}(\bar{y}_2)},$$

granting more influence to the most precise estimate.

If the survey coverage substantially differs for the two programs, e.g., because of differences in the sampling frame, then the above composite estimator was adjusted by assigning weights based on precision as well as stream miles covered (see Roth et al. 1999; Vølstad et al. 2002; Korn and Graubard 1999).

The above methods could be used to estimate proportions of stream miles in a specific class by introducing an indicator variable that takes the value 1 when the variable *y* meets the condition (e.g., pH < 6), and zero otherwise. The mean of this indicator using the estimators above is an estimate of the proportion of stream miles within the specific class (e.g., proportion of stream miles with pH < 6). The estimation of exact confidence intervals for pooled data based on the binomial distribution (section 2.3.1.1) was valid only if the County program also employed simple random or an equivalent sampling design.

6.3.1.8 Testing for Differences in Mean IBI Scores Between Years

Comparisons of statistical differences between mean IBI scores from two years were conducted using the standard method recommended by Schenker and Gentleman (2001). This test was used because it is more robust than the commonly used method of examining the overlap between the two associated confidence intervals. Assume that $\hat{Q}_1$, and $\hat{Q}_2$ are two independent estimates of mean

IBI, and that the associated standard errors (SE) are estimated by $\hat{S}E_1$ and $\hat{S}E_2$. We estimated the 95% confidence interval for $\hat{Q}_1 - \hat{Q}_2$ by

$$\left(\hat{Q}_1 - \hat{Q}_2\right) \pm 1.96\left[\hat{S}E_1^2 + \hat{S}E_2^2\right]^{1/2}$$

and tested (at 5% nominal level) the null hypothesis that $\hat{Q}_1 - \hat{Q}_2 = 0$ by examining whether the 95% confidence interval contains 0. The null hypothesis that two estimates are equal was rejected if and only if the interval did not contain 0 (Schenker and Gentleman 2001).

6.3.2 Estimators for Fish Populations

Fish populations were estimated by double-pass removal sampling using methods similar to those described by Heimbuch et al. (1997). The procedure corrects for bias introduced by variation in the probability of capture among sites to allow pooling of data over several sample sites. Such pooling can use data more effectively than traditional double-pass removal estimators for surveys with only two removal passes at individual sites because it reduces the number of estimates that fail when an equal or greater number of fish are captured on the second pass than on the first. For this analysis, data were pooled across samples within primary sampling units (PSUs), which were delineated by MDE 8-digit watersheds.

To estimate the abundance of a species, the number of fish captured on the first and second passes was pooled and then scaled to the total distance of streams within each PSU as:

$$\hat{x}_1 = \frac{M}{m}\sum_{s=1}^{m} x_{1s}$$

and,

$$\hat{x}_2 = \frac{M}{m}\sum_{s=1}^{m} x_{2s}$$

where x_{1s} and x_{2s} are the number of fish collected in the first and second passes in stream segment s (s = 1, 2, 3, …, m), m is the number of 75-m stream segments sampled, and M is the number of 75-m segments within the PSU as estimated using MDE 8-digit maps.

The bias-corrected estimator of abundance within a PSU ($\tilde{N}^*$) was calculated as:

$$\tilde{N}^* = \frac{\hat{x}_1^{\,2}}{(\hat{x}_1 - \hat{x}_2)}(1 + \hat{\gamma}^2),$$

where

$$\hat{\gamma}^2 = \frac{\frac{1}{m*}\sum_{s=1}^{m} \hat{p}_s^{\,2}\delta_s}{\left(\frac{1}{m*}\sum_{s=1}^{m} \hat{p}_s\delta_s\right)^2} - 1,$$

$$\hat{p}_s = 1 - \left(\frac{x_{2s}}{x_{1s} + 1}\right),$$

and $\delta_s = 1$ if $0 \le \hat{p}_s \le 1$ or 0 otherwise, and

$$m* = \sum_{s=1}^{m} \delta_s .$$

We made two modifications to the procedure described by Heimbuch et al. (1997). First, if an estimate failed (i.e., $\hat{x}_2 \ge \hat{x}_1$), then $\tilde{N}^*$ was assigned the total number of fish captured, scaled to the total stream distance in the PSU ($\hat{x}_2 + \hat{x}_1$). Second, final estimates of abundance and associated variance were calculated using the jackknife procedure. The jackknife consisted of calculating the estimates a number of times equal to the number of segments sampled, but omitting one observation from each calculation. The jackknife mean and variance were then calculated as described by Efron (1982).

6.4 COUNTY CONDITIONS ANALYSIS

6.4.1 County Biodiversity Ranking Map Preparation

To provide counties with biodiversity information directly relevant to watershed management and stream restoration and protection, biodiversity maps for each county and Baltimore City were prepared using the biodiversity ranking approach described in section 6.5.3. Each PSU (Primary Sampling Unit) within a county was color-coded according to its tier category. In addition, reaches were color-coded according to whether they had IBI scores greater than 4.2 (above the minimum for the Good range), or whether they had observed vs. expected (O/E) values for fish that exceeded 0.75 (i.e., greater than three fourths of predicted species were actually observed; see Stranko et al. in press). All reaches with species of greatest conservation need (GCN) were shown as well, with non-state listed GCN species shown separate from listed species. Finally, the 12-digit subwatershed or subwater-sheds associated with listed species in its stronghold watershed was also identified. This approach was used to ensure that sufficient area surrounding a site where a rare species was collected would be protected and to ensure that the exact location where a species was found could not be readily determined.

Because most stream reaches in a county were not sampled, it is likely that some stream reaches contain GCN species and/or high IBI or O/E scores. To provide a means for locating additional areas of high biodiversity, sample site locations where no biodiversity indicators were identified were also mapped.

6.5 BIODIVERSITY ANALYSIS

6.5.1 Derivation of Distinct Fish Assemblage Types

Data from 843 sites sampled by the MBSS (1995-1997) were used in the analysis of stream fish assemblages in Maryland. These 843 randomly-selected sites were located in 17 of the major drainage basins, and in all physiographic provinces in Maryland, providing sufficient coverage of the state. These sites represented the entire spectrum of stream conditions, ranging in quality from relatively pristine to severely degraded by anthropogenic disturbance. This MBSS database included quantitative data on 85 fish species collected statewide. Data on all native and non-native fish species were included, and rare species were not removed before analysis.

To account for the effect of stream size on stream fish assemblage composition and species abundance, MBSS sites were separated by stream order (Strahler 1957) prior to analysis. Stream order represents the longitudinal location of a stream within a watershed, and was therefore assumed to be a reliable surrogate for stream size. Generally, small stream communities differ from large stream communities in species composition and species abundances (Angermeier and Winston 1999). The dataset was initially partitioned by stream order to account for this variation. Sample sizes by stream order consisted of 247 first-order sites, 307 second-order sites, and 289 third-order sites.

The first stage in the characterization of fish assemblages in Maryland involved the classification of the 843 MBSS sites. Fish relative abundance data were analyzed by stream order with Ward's Minimum Variance Hierarchical Cluster Analysis using SAS software (SAS Institute 1999). With this technique, sample sites were assigned to cluster groups based upon similarities in species composition and relative abundances. Species present in 50% or more of the sites within a cluster were considered to be "member species" of that cluster. Based upon this 50% criterion, species lists were generated for each cluster. Comparisons were made between species lists for each cluster to identify distinct fish assemblages. Geographic distributions of clusters were examined using ArcView GIS software (ESRI 1998). This spatial analysis aided in interpretation of cluster results and facilitated the identification and description of fish assemblage types.

Cluster dendrograms, species composition, and geo-graphic distribution of clusters were used to determine which clusters represented distinct fish assemblages. Nineteen abiotic variables considered important to stream biota were used in examining abiotic characteristics of assemblages. Chemical variables used in the analysis were dissolved oxygen, pH, conductivity, acid neu-tralizing capacity (ANC), dissolved organic carbon (DOC), nitrate, and sulfate. Physical variables used included stream temperature, instream habitat quality, velocity/depth diversity, pool/glide/eddy quality, riffle/run quality, percent substrate embeddedness, and average thalweg depth. Upstream catchment area, percent urban land use, percent agricultural land use, percent forested land use, and altitude were watershed variables used in this analysis. Data on these 19, abiotic variables were analyzed using ANOVA Duncan's Multiple Range Test ($p < 0.05$). Variables not normally distributed were transformed prior to analysis.

This classification yielded distinct fish assemblages in first-, second-, and third-order streams that were unique in species composition and geographic distribution. These assemblages differed in many abiotic characteristics as well, suggesting that assemblage spatial variability was related to environmental and land use gradients occurring across the state. To examine these gradients in greater detail, it was necessary to complete the second stage of assemblage characterization. Ordination of data from the 843 MBSS sample sites was used to validate classification results and to examine environmental factors important in determining the composition and geographic distribution of the sixteen fish assemblage types.

Detrended Correspondence Analysis (DCA) is a robust ordination procedure that arranges community data in a manner that reduces problems of distortion and axis compression common among other ordination techniques (Gauch 1982). These adjustments facilitate subsequent interpretation of community patterns along environmental gradients. DCA was used to ordinate sites based on fish relative abundance data. All DCA analyses were performed using PC-ORD software (McCune and Medford 1997). DCA was performed at two spatial scales in all stream orders. Inter-regional DCA analysis was used to investigate statewide assemblage spatial variability and environmental gradients influencing the composition and distribution of assemblages. Intra-regional DCA analysis examined assemblages and environmental gradients existing within the non-Coastal Plain and Coastal Plain regions of Maryland. A visual assessment of the DCA ordination graphs was used to determine the degree of correspondence between groupings of sites in the ordinations and the assemblages identified by cluster analysis. Environmental gradients influencing assemblage structure at inter- and intra-regional scales were identified by Pearson correlations between DCA axis scores and site-specific environmental variables using SAS software (SAS Institute 1999). Site scores generated for each DCA axis were correlated with data on chemical, physical, and landscape variables. The correlations between DCA site scores and environmental variables ($p < 0.05$) were used to quantify the relationships between fish assemblage composition and geographic distribution along environmental gradients existing statewide and within the non-Coastal Plain and Coastal Plain regions of Maryland.

6.5.2 Definition of High and Low Integrity Streams

High and low integrity sites were identified using modified criteria from the MBSS sentinel site network and IBI development. High integrity sites were first determined as those with a Combined Biotic Index (CBI) score greater than 4.0. The resulting sites were then required to meet all of the following landuse, water quality, and habitat criteria:

- Forest Land Use >75.0%
- SO_4 < 50.0 mg/L
- pH > 6.0 or Dissolved Organic Carbon >8.0 mg/L
- NO_3 < 4.0 mg/L
- Dissolved Oxygen > 5.0 mg/L
- Riparian Width ≥ 50 m
- Instream Habitat Score > 15

Low integrity sites were defined as those with a CBI score less than 2.0, and meeting any of the criteria below:

- pH ≤ 5.0 and ANC ≤ 0 μeq/L
- Dissolved Oxygen ≤ 2.0 mg/L
- NO3 > 7.0 mg/L and Dissolved Oxygen < 3.0 mg/L
- Instream Habitat Score ≥ 5 and Urban Land Use > 50%
- Urban Land Use > 50% and Riparian Width = 0m

6.5.3 Biodiversity Ranking

A sequential approach was used to develop a stream biodiversity ranking for Maryland. First, one stronghold watershed (PSU watershed used for MBSS 2000-2004 sampling; see Table 6-1) was determined for each state listed and non-listed GCN (species identified by DNR as being of greatest conservation need) fish, stream salamander, or freshwater mussel species. Species not collected in the past ten years despite intensive searching (e.g. Maryland darter) were not used in the biodiversity ranking (Table 6-4; list of GCN fish, herps, mussels). The determination of stronghold status was based on a combination of MBSS quantitative and qualitative data, other data sources where available, and best professional judgment. All PSUs used as watersheds for MBSS sampling that were a stronghold for one or more state-listed fish, stream salamander, or freshwater mussel species were considered Tier 1 - the most important group in the Aquatic Biodiversity Ranking system. PSUs that were strongholds for non-state listed GCN species but also had state-listed GCN species present were classified as Tier 2 watersheds - the second highest priority in the freshwater biodiversity ranking. Tier 3 consisted of those watersheds that were strongholds for non-state listed GCN species and did not contain state-listed fish, stream salamanders, or freshwater mussels. Of the remaining watersheds, those with state-listed aquatic species present (but were not strongholds for state-listed species) were classified as Tier 4. Further classification was made by including those watersheds necessary to conserve all native fishes, freshwater mussels, stream and riverine herpetofauna, and rare, pollution-sensitive benthic macroinvertebrates (Tier 5) (Table 6-5); known as Biological Conservation Units (BCUs). The remaining watersheds were classified as Tier 6, the lowest ranking for freshwater biodiversity.

Table 6-4. List of fishes, herpetofauna, and freshwater mussel species used in freshwater biodiversity ranking.			
	Scientific Name	**Common Name**	**State Listed**
Fishes			
	Lampetra aepyptera	Least brook lamprey	
	Lampetra appendix	American brook lamprey	X
	Cottus bairdi	Mottled sculpin	
	Cottus sp 7	Checkered sculpin	
	Lepisosteus osseus	Longnose gar	
	Salvelinus fontinalis	Brook trout	
	Clinostomus funduloides	Rosyside dace	
	Notropis amoenus	Comely shiner	X
	Notropis chalybaeus	Ironcolor shiner	X
	Luxilus chrysocephalus	Striped shiner	X
	Margariscus margarita	Pearl dace	X
	Ericymba buccata	Silverjaw minnow	
	Hypentelium nigricans	Northern hogsucker	
	Noturus flavus	Stonecat	X
	Ameiurus catus	White catfish	
	Acantharchus pomotis	Mud sunfish	X
	Centrarchus macropterus	Flier	X
	Enneacanthus gloriosus	Bluespotted sunfish	
	Enneacanthus obesus	Banded sunfish	
	Lepomis gulosus	Warmouth	
	Etheostoma blennioides	Greenside darter	
	Etheostoma fusiforme	Swamp darter	X
	Etheostoma nigrum	Johnny darter	
	Etheostoma vitreum	Glassy darter	X
	Percina caprodes	Logperch	X
	Percina notogramma	Stripeback darter	X
	Percina peltata	Shield darter	
Amphibians			
	Cryptobranchus alleganiensis	Hellbender	X
	Desmognathus monticola	Seal salamander	
	Desmognathus ochrophaeus	Mountain dusky salamander	
	Eurycea longicauda	Longtail salamander	
	Pseudotriton montanus	Mud salamander	
	Pseudotriton ruber	Red salamander	
	Necturus maculosus	Mudpuppy	X
Reptiles			
	Glyptemys insculpta	Wood turtle	
	Graptemys geographica	Map turtle	X
	Apalone spinifera	Eastern spiny softshell	X
	Farancia erytrogramma	Rainbow snake	X
	Nerodia erythrogaster erythrogaster	Redbelly water snake	X
	Regina septemvittata	Queen snake	

Table 6-4. (Continued)			
	Scientific Name	**Common Name**	**State Listed**
Freshwater Mussels			
	Alasmidonta heterodon	Dwarf wedge mussel	X
	Alasmidonta undulata	Triangle floater	X
	Alasmidonta varicosa	Brook floater	X
	Anodonta implicata	Alewife floater	
	Elliptio fisheriana	Northern lance	
	Elliptio lanceolata	Yellow lance	
	Elliptio producta	Atlantic spike	
	Lampsilis cariosa	Yellow lampmussel	X
	Lampsilis radiata	Eastern lampmussel	
	Lasmigona subviridis	Green floater	X
	Leptodea ochracea	Tidewater mucket	
	Ligumia nasuta	Eastern pondmussel	
	Strophitus undulatus	Squawfoot	X
	Utterbackia imbecillis	Paper pondshell	

To rank individual watersheds within each tier, three metrics were combined with equal weighting. These metrics were migratory fish density, fish assemblage intactness, and a rarity weighting. To provide a means for equal weighting, all scores for each metric were rescaled to have values from 0 to 1.

Quantitative, two pass MBSS electrofishing data were used to construct a migratory fish metric. Because MBSS fish sampling is done in summer, a number of migratory fish species are under-sampled or not collected at all. However, comprehensive information on migratory fish usage among PSUs does not exist, so MBSS data were used as an indicator of migratory fish density. A total of ten species collected by MBSS were considered in the overall migratory fish density estimate (Table 6-6; list of migratory fish species used for biodiversity ranking).

The number of migratory fish per stream mile in a PSU was used to construct the metric. An exception was watersheds upstream from Great Falls on the Potomac River. These watersheds were assigned a score of zero even though some had low densities of American eel present. This decision was made because anadromous fish are not able to pass above Great Falls, and the metric was intended to be a surrogate for all migratory fish species. The purpose of the migratory fish metric was to recognize the vital importance of migratory fishes to the biodiversity and ecology of streams and rivers in Maryland.

MBSS data were also used for the fish assemblage intactness metric. This metric was based on the density-adjusted number of sites with high IBI (fish or benthic macroinvertebrates) scores (IBI greater than or equal to 4.2) or high O/E scores (O/E greater than or equal to 0.75). Density values were then rescaled to fit a 0 to 1 scale. The purpose of this metric was to recognize the importance of higher integrity watersheds that may or may not have rare species present.

The third metric used to rank watersheds within their respective tiers, rarity, was also calculated using MBSS data. To compile this index, all fish, freshwater mussels, and stream salamanders were used. For each species, the inverse of the number of watersheds in which the species occurred (out of 84 PSUs) was calculated. The resulting values were summed for each watershed and scaled from 0-1. This calculation ensured that watersheds with the highest numbers of infrequently occurring species had higher rarity values.

6.5.4 PSU Threats

The extent, severity, persistence, trend, and reversibility of 20 different threats to the biological integrity of freshwater streams were determined for each 8-digit watershed in Maryland. A total of 20 threat indices were grouped into four major categories (Non-native species, Chemical, Habitat alteration, and Future changes) and ranked on a scale from zero to five (see Volume 9: Stream and River Biodiversity). Threat indices and associated rankings were based on a combination of MBSS, along with state and federal data sources and best professional judgment.

Table 6-5. List of Sensitive, rare benthic macroinvertebrate taxa used to establish biodiversity conservation units (BCUs) as part of the freshwater biodiversity ranking process.

Order	Family	Genus	Common Names for orders and families
Ephemeroptera			Mayflies
	Baetidae		small minnow mayflies
		Cloeon	
		Barbaetis	
	Ephemerellidae		spiny crawler mayflies
		Attenella	
		Timpanoga	
	Ephemeridae		common burrower mayflies
		Litobrancha	
		Pentagenia	
	Heptageniidae		flathead mayflies
		Nixe	
	Potamanthidae		hacklegill mayflies
		Anthopotamus	
Odonata			Dragonflies
	Aeshnidae		darner dragonflies
		Aeshna	
		Anax	
		Nasiaeschna	
	Gomphidae		clubtail dragonflies
		Arigomphus	
		Erpetogomphus	
	Lestidae		spreadwinged damselflies
		Lestes	
	Libellulidae		skimmer dragonflies
		Erythemis	
		Leucorrhinia	
		Libellula	
		Pachydiplax	
		Plathemis	
Plecoptera			Stoneflies
	Capniidae		small winter stoneflies
		Capnia	
	Chloroperlidae		green stoneflies
		Perlinella	
	Nemouridae		nemourid stoneflies
		Paranemoura	
		Shipsa	
	Perlodidae		perlodid stoneflies
		Helopicus	
		Yugus	

Table 6-5. (Continued)

Order	Family	Genus	Common Names for orders and families
Trichoptera			Caddisflies
	Calamoceratidae		comblipped case maker
		Anisocentropus	
	Helicopsychidae		snailcase maker caddisflies
		Helicopsyche	
	Hydropsychidae		common netspinner caddisflies
		Potamyia	
	Hydroptilidae		micro caddisflies
		Ochrotrichia	
		Orthotrichia	
	Phryganeidae		giant casemaker caddisflies
		Oligostomis	
	Dytiscidae		predaceous diving beetles
		Coptotomus	
		Derovatellus	
		Helocombus	
		Hydaticus	
		Uvarus	
	Hydrophilidae		water scavenger beetles
		Helochares	
Diptera			True flies
	Ceratopogonidae		biting midges
		Atrichopogon	
		Alluaudomyia	
	Chironomidae		non-biting midges
		Cryptotendipes	
		Xenochironomus	
		Psectrotanypus	
		Georthocladius	
		Metriocnemus	
		Pseudosmittia	
		Guttipelopia	
	Simuliidae		black flies
		Greniera	
	Tanyderidae		primative crane flies
		Protoplasa	
	Tipulidae		craneflies
		Liogma	

Table 6-6. List of migratory fish species used for biodiversity ranking			
Order	**Family**	**Scientific Name**	**Common Name**
Petromyzontiformes	Petromyzontidae	*Petromyzon marinus*	sea lamprey
Anguilliformes	Anguillidae	*Anguilla rostrata*	American eel
Clupeiformes	Clupeidae	*Alosa aestivalis*	blueback herring
Clupeiformes	Clupeidae	*Alosa mediocris*	hickory shad
Clupeiformes	Clupeidae	*Alosa pseudoharengus*	alewife
Clupeiformes	Clupeidae	*Alosa sapidissima*	American shad
Siluriformes	Ictaluridae	*Ameiurus catus*	white catfish
Perciformes	Moronidae	*Morone americana*	white perch
Perciformes	Moronidae	*Morone saxatilis*	striped bass
Perciformes	Percidae	*Perca flavenscens*	yellow perch

6.5.4.1 Non-native or Invasive Species

MBSS data for invasive plants within a 50-m riparian buffer were compiled for each watershed. The presence and extent of multiflora rose, tearthumb, Japanese honeysuckle, Japanese stilt grass, and thistle was determined, standardized by the number of miles of fresh-water streams in each watershed, and ranked according to the percentile to determine extent.

The number of MBSS sites with non-native fishes was determined and weighted by the abundance of non-native fishes. The weighted number of sites was then standardized by the number of miles of freshwater streams in each watershed. The presence of *Corbicula fluminea* (the Asiatic clam) and *Orconectes virilis* (an introduced crayfish) at MBSS sites was used to calculate the percentage of sites in each watershed. These data were combined with the non-native fish species and ranked according to percentile to determine extent.

6.5.4.2 Chemical

- Low Dissolved Oxygen. The extent of streams with low dissolved oxygen concentrations was determined using MBSS water chemistry data. The percentage of sites in a watershed with dissolved oxygen concen-trations below 4.5 mg/L were determined and ranked for each watershed. Sites meeting conditions con-sidered to be blackwater (Southerland et al. 2005) were considered to exhibit naturally low dissolved oxygen concentrations and were not used in the calculation of this index.

6.5.4.3 Non-point Sources

- Excess Nitrogen. Excess nitrogen loading to streams was determined using MBSS water chemistry data. The percentage of sites with nitrate concentrations above 5.0 mg/L was determined and ranked for each watershed. Nitrate ion wet deposition data from 1998-2003 (NADP 2005) were also obtained and used to rank watersheds in Maryland. The MBSS data contained a multitude of data points and appeared to provide a finer resolution of detail than the NADP data. For this reason, rankings-based MBSS data were used to rank watersheds using the percentile rankings described in Volume 9: Stream and Riverine Biodiversity.

- Excess Phosphorus. Excess phosphorus loading to streams was determined using MBSS water chemistry data. The percentage of sites with total phosphorus concentrations above 0.2 mg/L was determined and ranked for each watershed using rankings described in Volume 9: Stream and Riverine Biodiversity.

- Agricultural Pesticides. The amount of agricultural pesticide usage for each watershed was estimated based on the percentage of randomly-selected MBSS sites containing greater than 50% of agricultural land usage in the site catchment area. These data were then ranked according to percentile to determine extent.

- Acid Deposition/Low pH. The proportion of streams where low pH was measured and attributed to atmospheric deposition was calculated for each watershed and ranked. Acid deposition was considered to be the causal factor when ANC < 200 μeq/L and sulfate concentrations < 500 μeq/L (Roth et al. 1996).

- Mercury Deposition. Total mercury wet deposition data from 1998-2003 (NADP 2005) was summarized for watersheds in Maryland. These data were ranked according to percentile to determine extent.

- Acid Mine Drainage. Acid mine drainage was considered to be the causal factor when ANC < 200 μeq/L and sulfate concentrations > 300 μeq/L (Roth et al. 1996). The proportion of sites where low pH was recorded and attributed to acid mine drainage was calculated for each watershed and ranked as described in Volume 9: Stream and Riverine Biodiversity.

6.5.4.4 Point Sources

- Pathogens/Endocrine Disruptors. Threats from contaminants related to wastewater treatment facilities and sewage leaks, such as endocrine disrupters, antibiotics, and pathogens were estimated using data for municipal NPDES discharges and by determining the percentage of watersheds covered by sewage service. Heavier weights were applied to older systems, larger facilities, and areas with documented infrastructure problems. These data were ranked according to percentile to determine extent.

- Industrial (NPDES) Discharges. The number of permitted discharges from industrial NPDES sites was summarized for each watershed, with heavier weights applied to larger facilities. These data were standardized by watershed area and ranked according to percentile to determine extent.

- Organic Matter Retention. The inability to retain natural organic matter because of habitat deficiency (woody debris, natural sinuosity), and increased discharge related to storm flow from increased impervious surface was estimated for each watershed. The percentage of MBSS sites with straight-line distances greater than 70m (93.3% straight) that contained no woody debris was ranked according to percentile to determine extent. In order to avoid misclassifying lower order steep streams in the highlands, sites with gradient measurements greater than 4% were excluded from this analysis.

6.5.4.5 Habitat Alteration

- Channelization. The proportion of MBSS sites in each watershed that were channelized (tax ditches, flood control) was calculated and ranked as described in Volume 9: Stream and Riverine Biodiversity.

- Forest Fragmentation. Four indicators of forest fragmentation (percent forest, average size of forested patches, percent of interior forest, and the length of forested edge) were determined for each watershed by the Chesapeake Coastal Watershed Service (2000). These indicators were standardized by watershed area and ranked according to percentile to determine extent.

- Migration Barriers. The number of barriers (or blockages) to fish migration was determined for each watershed by performing a GIS intersection of roads and streams. This information was combined with the Maryland DNR Fisheries Service (2001) 'fish blockages' GIS by watershed. These data were standardized by watershed area and ranked according to percentile to determine watersheds with the highest concentration of fish blockages.

- Water Withdrawal. The total estimated daily use in millions of gallons per day (MGD) of surface and ground water from both urban and agricultural sources (Smith 1999) was summarized for each watershed and ranked according to percentile to determine extent.

- Road Density. The density of road networks was summarized for each watershed using the 1:100,000 scale TIGER road dataset (Redistricting Census, 2000). Roads were weighted according to width (traffic capacity), standardized by watershed area, and ranked according to percentile to determine extent.

- Sedimentation. Sediment loadings were estimated for each watershed by the weighted proportion of MBSS sites with large percentages of unstable, eroded banks in each watershed. The percentiles were then ranked as described in Volume 9: Stream and Riverine Biodiversity.

- Runoff/Baseflow. Threats related to runoff and modified baseflow conditions were estimated for each watershed using an index of population density (Maryland Department of Planning, 2004) and a weighted proportion of MBSS sites with riparian buffers less than 5 meters or containing buffer breaks.

These data were summarized for each watershed and ranked according to percentile to determine extent.

- Wetland Loss. The estimated acreage of historical wetland loss (i.e., non-wetland hydric soils) was summarized by the Clean Water Action Plan Technical Workgroup (1998) for each watershed and ranked according to percentile to determine extent.

6.5.4.6 Future Changes

- Sea Level Rise. Threats to freshwater streams due to sea level rise were determined by weighting watersheds with lands close to sea level using thresholds (below 1.5 m, 1.5 to 3.5 m, and 3.5 to 4 m) determined by Johnson (2000) and Titus (1998).

- Land Conversion. Population growth data was obtained from the Maryland Department of Planning, 2004. The projected population in 2030 for each county in Maryland was compared to 2005 population numbers and a percentage change was calculated. The county projections were then overlaid with and applied to each watershed in the county. When watersheds spanned more than one county, data from the county with the higher projection was applied to the watershed.

6.6 LANDOWNER PERMISSION RESULTS

As discussed in Section 6.2.4, permissions were obtained to access privately owned land adjacent to or near each stream segment. For 2000-2004, the overall success rate for obtaining permissions was 63% (Table 6-7). Cases where permissions were not obtained included both denials (10%) as well as non-responses (26%), when landowners were unable to be reached and did not respond to letters and telephone messages. The success rate was 86% for landowners who responded to phone or letter permission requests. Reasons for permission denial varied widely and generally reflected the preferences of individual landowners regarding property access, rather than any specific types of land. In rare cases, permission denial may affect the interpretation of MBSS estimates, but only where denials occur in streams with characteristics that differ from the general population of streams. During 2000-2004 sampling, it did not appear that most permission denials affected MBSS estimates, although it was felt by field crews that permission denials in some PSUs may have resulted in more sites sampled on public lands than was proportionate to the amount of public land in the PSU. Additionally, two basins appear to be biased due to uniform denials from mining operations; North Branch Potomac and the Youghiogheny. For yearly permission rates, refer to the MBSS five-year QA report (Roth et al. 2005).

6.7 NUMBER OF SITES SAMPLED IN 2000-2004

As stated in Section 6.2.2.2 above, the target sample size in each PSU was a minimum of 10 sites for the spring benthic sampling. Additional sites were allocated to the larger PSUs sampled in 2000-2004 (Table 6-8). Table 6-9 lists the number of sites sampled for spring benthic, physical habitat, and water chemistry sampling. For each PSU, the number of sites actually sampled equaled or exceeded the target number specified in the design. Ninety-six sites were unsampleable in the spring (2000-2004) for a variety of reasons, including dry stream beds, beavers, and tidal influence. Note that in 2000, in both St. Mary’s River and Patapsco River Lower North Branch, one site was deemed unsampleable for benthos, but water quality and habitat measurements were made. In 2001, in both Assawoman/Isle of Wight/Sinepuxent/Newport/ Chincoteague Bays and Sassafras River/Stillpond-Fairlee, only nine sites were sampled instead of the targeted ten. This was due to an abundance of dry streams in each of the watersheds and/or lower than anticipated landowner permission rates in those PSUs. In addition, dry streams were a significant problem in 2002 due to statewide drought conditions.

Table 6-7. Landowner permission success rates for Primary Sampling Units (PSUs) sampled in the 2000-2004 MBSS

	Number of Stream Segments Targeted as Potential Sample Sites	Success Rate	No Response	Denial Rate
PSUs-2000				
Casselman River	26	69%	31%	0%
Town Creek	20	80%	15%	5%
Fifteen Mile Creek	20	90%	10%	0%
Potomac River Washington County/ Marsh Run/Tonoloway/ Little Tonoloway	24	84%	16%	0%
Upper Monocacy River	34	64%	25%	1%
Mattawoman Creek	18	61%	33%	6%
Nanjemoy Creek	20	55%	45%	0%
St. Mary's River	18	72%	17%	11%
Brighton Dam	26	62%	26%	12%
Little Patuxent River	26	81%	18%	1%
South Branch Patapsco River	22	60%	32%	8%
Liberty Reservoir	30	83%	7%	0%
Patapsco River Lower North Branch	28	71%	25%	4%
Prettyboy Reservoir	24	63%	25%	12%
Aberdeen Proving Ground/Swan Creek	20	65%	15%	20%
Corsica River/Southeast Creek	20	74%	16%	10%
Upper Choptank	26	54%	23%	23%
Lower Wicomico River/Monie Bay/ Wicomico Creek/Wicomico River Head	25	56%	32%	12%
PSUs-2001				
Youghiogheny River	32	60%	15%	25%
Potomac River Upper North Branch	20	90%	5%	5%
Potomac Allegany County/Sideling Hill Creek	20	90%	5%	5%
Seneca Creek	30	63%	27%	10%
Piscataway Creek	20	75%	20%	5%
Potomac Upper Tidal/Oxon Creek	20	65%	30%	5%
Zekiah Swamp	26	69%	31%	0%
Gilbert Swamp	20	70%	20%	10%
Assawoman/Isle of Wight/Sinepuxent/Newport/ Chincoteague Bays	20	45%	35%	20%
Western Branch	20	60%	40%	0%
Patuxent River Middle	26	58%	27%	15%
Bodkin Creek/Baltimore Harbor	20	90%	10%	0%
Little Gunpowder Falls	20	65%	30%	5%
Sassafras River/Stillpond-Fairlee	20	75%	15%	10%
Northeast River/Furnace Bay	20	55%	35%	10%
Nanticoke River	20	70%	15%	15%
Dividing Creek/Nassawango Creek	20	60%	35%	5%
Upper Pocomoke River	26	69%	19%	12%
Deer Creek	28	75%	14%	11%

Table 6-7. (Continued)				
	Number of Stream Segments Targeted as Potential Sample Sites	**Success Rate**	**No Response**	**Denial Rate**
PSUs-2002				
Back River	20	90%	10%	0%
Breton/St. Clements Bays	20	70%	20%	10%
Conewago Creek/Double Pipe Creek	34	71%	23%	6%
Conococheague	22	82%	13%	5%
Eastern Bay/Kent Narrows/Lower Chester River/ Langford Creek/Kent Island Bay	30	50%	30%	20%
Gunpowder River/Lower Gunpowder Falls/ Bird River/ Middle River-Browns	20	75%	25%	0%
Jones Falls	20	70%	5%	25%
Loch Raven Reservoir	34	53%	44%	3%
Lower Pocomoke	20	65%	10%	25%
Middle Chester River	20	70%	20%	10%
Middle Patuxent River	20	60%	40%	0%
Nanticoke River	30	50%	40%	10%
Potomac River Lower Tidal/Potomac River Middle Tidal	20	65%	45%	0%
Potomac River Montgomery County	30	66%	24%	10%
Potomac River Washington County/Marsh Run/ Tonoloway/Little Tonoloway	36	50%	42%	8%
Rocky Gorge Dam	20	60%	35%	5%
Savage River	29	72%	20%	8%
South River/West River	20	80%	15%	5%
Town Creek	20	80%	10%	10%
PSUs-2003				
Potomac River Lower North Branch	30	60%	33%	7%
Georges Creek	20	55%	40%	5%
Antietam Creek	30	53%	34%	13%
Lower Monocacy	40	50%	30%	20%
Catoctin Creek	20	60%	10%	30%
Rock Creek/Cabin John Creek	20	60%	40%	0%
Liberty Reservoir	30	50%	50%	0%
St. Mary’s River	20	60%	20%	15%
Magothy/Severn Rivers	20	75%	25%	0%
Port Tobacco River	20	50%	40%	10%
West Chesapeake Bay	20	55%	45%	0%
Little Gunpowder Falls	20	60%	15%	25%
Broad Creek	20	50%	0%	50%
Lower Elk River PSU	20	55%	45%	0%
Miles/Wye Rivers	20	50%	50%	0%
Middle Chester River	20	70%	25%	5%
Honga River PSU	30	33%	33%	33%
Tuckahoe Creek	20	50%	30%	20%
Pocomoke Sound PSU	20	55%	15%	30%

Table 6-7. (Continued)				
	Number of Stream Segments Targeted as Potential Sample Sites	**Success Rate**	**No Response**	**Denial Rate**
PSUs-2004				
Anacostia River	30	57%	23%	20%
Bodkin Creek/Baltimore Harbor	28	57%	28%	14%
Bush River/Bynum Run	20	65%	20%	15%
Deer Creek	38	47%	36%	16%
Evitts Creek	20	80%	15%	5%
Fishing Bay/Transquaking River	20	60%	35%	5%
Gwynns Falls	36	67%	22%	11%
Little Conococheague/Licking Creek	25	48%	32%	20%
Little Youghiogheny/Deep Creek Lake	20	60%	15%	25%
Lower Susquehanna River/ Octoraro Creek/Conowingo Dam	28	43%	43%	14%
Lower Winters Run/Atkisson Reservoir	20	65%	20%	15%
Marshyhope Creek	20	60%	25%	15%
Patuxent River Lower	34	47%	38%	15%
Patuxent River Upper	22	59%	41%	5%
Potomac Middle Tidal/Potomac Lower Tidal	20	65%	30%	5%
Potomac River Frederick County	19	74%	16%	11%
Upper Chester River	20	70%	30%	0%
Wicomico River	20	55%	25%	20%
Wills Creek	18	61%	28%	11%

Table 6-8. Number of extra sites sampled for larger PSUs for the 2000-2004 MBSS	
PSU	**Number of Extra Sites Sampled**
2000	
Upper Monocacy River	8
Liberty Reservoir	5
Patapsco River Lower North Branch	4
Upper Choptank	4
Little Patuxent River	3
Potomac River Washington County/Marsh Run/Tonoloway/ Little Tonoloway	3
2001	
Youghiogheny River	6
Seneca Creek	5
Deer Creek	4
Zekiah Swamp	3
Patuxent River Middle	3
Upper Pocomoke River	3
2002	
Conewago Creek/Double Pipe Creek	7
Loch Raven Reservoir	7
Savage River	7
Potomac River Montgomery County	5
Potomac River Washington County/Marsh Run/ Ronoloway/Little Tonoloway	3

Table 6-8. (Continued)	
PSU	**Number of Extra Sites Sampled**
2003	
Lower Monocacy River	11
Potomac River Lower North Branch	5
Liberty Reservoir	5
Antietam Creek	4
Catoctin Creek	4
2004	
Anacostia River	5
Lower Susquehanna/Octoraro Creek/Conowingo Dam Susquehanna River	1
Patuxent River Lower	10
Deer Creek	4

Table 6-9. Number of sites sampleable in the spring for MBSS 2000-2004 PSUs				
	Number of Unsampleable Sites	**Number of Benthic Sites**	**Number of Spring Habitat Sites**	**Number of Spring Water Quality Sites**
PSU 2000				
Casselman River	0	10	10	10
Town Creek	0	10	10	10
Fifteen Mile Creek	0	10	10	10
Potomac River Washington County/ Marsh Run/Tonoloway/ Little Tonoloway	3	13	13	13
Upper Monocacy River	3	18	18	18
Mattawoman Creek	0	11	11	11
Nanjemoy Creek	1	10	10	10
St. Mary's River	1	10	11	11
Brighton Dam	0	11	11	11
Little Patuxent River	1	13	13	13
South Branch Patapsco River	1	10	10	10
Liberty Reservoir	0	16	16	16
Patapsco River Lower North Branch	2	14	15	15
Prettyboy Reservoir	0	10	10	10
Aberdeen Proving Ground/Swan Creek	2	11	11	11
Corsica River/Southeast Creek	0	10	10	10
Upper Choptank	0	14	14	14
Lower Wicomico River/Monie Bay/Wicomico Creek/ Wicomico River Head	1	10	10	10
TOTAL	15	211	213	213

Table 6-9. (Continued)	Number of Unsampleable Sites	Number of Benthic Sites	Number of Spring Habitat Sites	Number of Spring Water Quality Sites
PSU 2001				
Youghiogheny River	0	16	16	16
Potomac River Upper North Branch	0	10	10	10
Potomac AL Co/Sideling Hill Creek	0	10	10	10
Seneca Creek	0	15	15	15
Piscataway Creek	0	10	10	10
Potomac Upper Tidal/Oxon Creek	0	10	10	10
Zekiah Swamp	0	13	13	13
Gilbert Swamp	0	10	10	10
Assawoman/Isle of Wight/Sinepuxent/Newport/ Chincoteague Bays	0	9	9	9
Western Branch	0	10	10	10
Patuxent River Middle	0	13	13	13
Bodkin Creek/Baltimore Harbor	3	10	10	10
Little Gunpowder Falls	0	10	10	10
Sassafras River/Stillpond-Fairlee	6	9	9	9
Northeast River/Furnace Bay	0	10	10	10
Nanticoke River	0	10	10	10
Dividing Creek/Nassawango Creek	0	10	10	10
Upper Pocomoke River	0	13	13	13
Deer Creek	0	14	14	14
TOTAL	9	212	212	212
PSU 2002				
Back River	1	10	10	10
Breton/St. Clements Bays	2	10	10	10
Conewago Creek/Double Pipe Creek	2	17	17	17
Conococheague	8	10	10	10
Eastern Bay/Kent Narrows/Lower Chester River/Langford Creek/Kent Island Bay	3	10	10	10
Gunpowder River/Lower Gunpowder Falls/ Bird River/Middle River-Browns	0	10	10	10
Jones Falls	0	10	10	10
Loch Raven Reservoir	0	17	17	17
Lower Pocomoke	3	10	10	10
Middle Chester River	4	10	10	10
Middle Patuxent River	0	10	10	10
Nanticoke River	2	10	10	10
Potomac River Lower Tidal/Potomac River Middle Tidal	2	10	10	10
Potomac River Montgomery County	2	15	15	15
Potomac River Washington County/Marsh Run/ Tonoloway/Little Tonoloway	5	13	13	13
Rocky Gorge Dam	0	10	10	10
Savage River	0	17	17	17
South River/West River	1	10	10	10
Town Creek	3	10	10	10
TOTAL	38	219	219	219

Table 6-9. (Continued)				
	Number of Unsampleable Sites	**Number of Benthic Sites**	**Number of Spring Habitat Sites**	**Number of Spring Water Quality Sites**
PSU 2003				
Potomac River Lower North Branch	2	15	15	15
Georges Creek	0	10	10	10
Antietam Creek	4	14	14	14
Lower Monocacy	3	21	21	21
Catoctin Creek	0	14	14	14
Rock Creek/Cabin John Creek	0	10	10	10
Liberty Reservoir	1	15	15	15
St. Mary’s River	1	10	10	10
Magothy/Severn Rivers	0	10	10	10
Port Tobacco River	0	10	10	10
West Chesapeake Bay	1	10	10	10
Little Gunpowder Falls	0	10	10	10
Broad Creek	1	10	10	10
Lower Elk River PSU	2	10	10	10
Miles/Wye Rivers	0	10	10	10
Middle Chester River	5	10	10	10
Honga River PSU	0	10	10	10
Tuckahoe Creek	1	10	10	10
Pocomoke Sound PSU	0	10	10	10
TOTAL	21	219	219	219
PSU 2004				
Little Youghiogheny/Deep Creek Lake	0	10	10	10
Evitts Creek	1	10	10	10
Wills Creek	0	10	10	10
Little Conococheague/Licking Creek	0	10	10	10
Potomac River (Frederick County)	0	10	10	10
Anacostia River	0	15	15	15
Bodkin Creek/Baltimore Harbor	3	10	10	10
Gwynns Falls	0	10	10	10
Patuxent River (Lower)	0	20	20	20
Patuxent River (Upper)	0	10	10	10
Potomac Lower Tidal/Potomac Middle Tidal	4	10	10	10
Wicomico River	1	10	10	10
Lower Susquehanna/Octoraro Creek/Conowingo Dam Susquehanna River	0	11	11	11
Deer Creek	0	14	14	14
Lower Winters Run/Atkisson Reservoir	0	10	10	10
Bush River/Bynum Run	0	10	10	10
Upper Chester River	0	10	10	10
Marshyhope Creek	1	10	10	10
Fishing Bay/Transquaking River	2	10	10	10
TOTAL	12	210	210	210

During summer sampling, a number of sites that had been sampled in the spring were unsampleable for several reasons, the most common being that the stream had dried up. Table 6-10 lists the number of sites that were electrofished during the summer of 2000-2004. It also lists the number of sites where summer habitat and water quality measures were taken, as well as the number of sites where amphibians and reptiles, mussels, and aquatic vegetation were qualitatively sampled.

6.8 INDICATORS

An objective of the MBSS is to assess the status and trends in biological integrity for all 1^{st} – 4^{th} order non-tidal stream miles in Maryland. Therefore, it is critical that the MBSS provide estimates of the biological condition of streams using indicators based on references of biological integrity. Karr and Dudley (1981) used reference condition as the basis for their definition of biological integrity, i.e., "the ability of an aquatic ecosystem to support and maintain a balanced, integrated, adaptive community of organisms having a species composition, diversity, and functional organization comparable to that of natural habitats in the region." It is also important that the MBSS provide a reference-based indicator of physical habitat condition. A brief description of these indicators follows.

6.8.1 Biological Indicators

Multi-metric Indices of Biotic Integrity (IBIs), originally developed by Karr et al. (1986), are the most common indicators of stream condition in use today. Most IBIs develop their expectations for the structure and function of biological assemblages from reference sites.

The MBSS developed the first fish (Roth et al. 1998) and benthic macroinvertebrate (Stribling et al. 1998) IBIs for Maryland in 1998. Subsequently, Roth et al. (2000) refined the Maryland fish IBI and Southerland et al. (2004) developed a stream salamander IBI for Maryland. These original Maryland IBIs have performed well, helping Maryland DNR and other agencies better manage State waters, and have produced dozens of assessments and research findings. At the same time, these IBIs have not adequately captured reference conditions for some classes of streams, specifically some geographic areas, smaller streams, coldwater streams, and blackwater streams. In these cases, either a more general IBI has been applied to two classes of streams (e.g., both Highlands and Piedmont streams) or no IBI has been applied (e.g., streams draining catchments of less than 300 acres, blackwater streams).

To better assess Maryland streams, new IBIs that perform better and apply to more stream classes were developed (Southerland et al. 2005). By 2005, the MBSS had completed sampling at approximately 2500 stream sites, more than doubling the number of sites that were available for the original IBI development.

The development of new fish and benthic macroinvertebrate IBIs was undertaken with the following goals:

- Increase confidence that the reference conditions used are minimally disturbed
- Better capture the full range of natural variation in reference condition across the geographic regions and stream types of Maryland
- Increase the sensitivity of IBIs by segregating variation into more classes of reference condition
- Evaluate alternative scoring methods that might improve the performance of IBIs

At the same time, development of the new IBIs took into account the following practical constraints:

- Only a finite number of reference sites have been sampled in Maryland, so fewer reference sites are available to characterize reference condition when a larger number of geographic or stream type classes are used
- IBIs developed for larger geographic or stream type classes will be less sensitive for distinguishing between reference condition and degraded condition

With these objectives and constraints in mind, the new fish and benthic macroinvertebrate IBIs for Maryland were developed following the same steps used to develop the original MBSS IBIs:

- Develop the database
- Identify reference and degraded sites
- Determine appropriate strata
- Test candidate metrics
- Test and validate indices

In addition, the effects of alternative metric scoring methods on metric and index performance were evaluated.

New fish IBIs were developed for the Coastal Plain, Eastern Piedmont, warmwater Highlands, and coldwater

Table 6-10. Number of sites sampleable in the summer for MBSS 2000-2004 PSUs						
	Number of Sites Fished	**Number of Summer Habitat Sites**	**Number of Summer Water Quality Sites**	**Number of Sites - Amphibians and Reptiles**	**Number of Sites - Mussels**	**Number of Sites - SAV**
PSU 2000						
Casselman River	10	10	10	10	10	10
Town Creek	8	8	8	9	8	8
Fifteen Mile Creek	8	8	8	10	8	8
Potomac River Washington County/Marsh Run/Tonoloway/ Little Tonoloway	12	12	12	13	12	12
Upper Monocacy River	17	17	17	18	17	17
Mattawoman Creek	10	10	10	10	10	10
Nanjemoy Creek	10	10	10	10	10	10
St. Mary's River	9	9	9	10	9	9
Brighton Dam	11	11	11	11	11	11
Little Patuxent River	13	13	13	13	13	13
South Branch Patapsco River	10	10	10	10	10	10
Liberty Reservoir	16	16	16	16	16	16
Patapsco River Lower North Branch	13	13	13	14	13	13
Prettyboy Reservoir	10	10	10	10	10	10
Aberdeen Proving Ground/ Swan Creek	9	9	9	10	9	9
Corsica River/Southeast Creek	10	10	10	10	10	10
Upper Choptank	13	13	14	14	14	14
Lower Wicomico River/ Monie Bay/Wicomico Creek/ Wicomico River Head	10	10	10	10	10	10
TOTAL	199	199	200	208	200	200
PSU 2001						
Youghiogheny River	16	16	16	16	16	16
Potomac River Upper North Branch	10	10	10	10	10	10
Potomac AL Co/Sideling Hill Creek	6	6	6	9	6	6
Seneca Creek	14	14	14	14	14	14
Piscataway Creek	10	10	10	10	10	10
Potomac Upper Tidal/Oxon Creek	10	10	10	10	10	10
Zekiah Swamp	13	13	13	13	13	13
Gilbert Swamp	10	10	10	10	10	10
Assawoman/Isle of Wight/ Sinepuxent/Newport/Chincoteague Bays	7	7	7	9	7	7
Western Branch	9	9	9	9	9	9
Patuxent River Middle	13	13	13	13	13	13
Bodkin Creek/Baltimore Harbor	10	10	10	10	10	10
Little Gunpowder Falls	10	10	10	10	10	10
Sassafras River/Stillpond-Fairlee	9	9	9	9	9	9
Northeast River/Furnace Bay	9	9	9	9	9	9
Nanticoke River	8	9	9	9	9	9
Dividing Creek/Nassawango Creek	9	9	9	10	9	9
Upper Pocomoke River	12	12	12	13	12	12
Deer Creek	14	14	14	14	14	14
TOTAL	199	200	200	207	200	200

Table 6-10. (Continued)						
	Number of Sites Fished	**Number of Summer Habitat Sites**	**Number of Summer Water Quality Sites**	**Number of Sites - Amphibians and Reptiles**	**Number of Sites - Mussels**	**Number of Sites - SAV**
PSU 2002						
Back River	10	10	10	10	10	10
Breton/St. Clements Bays	5	5	5	8	5	5
Conewago Creek/Double Pipe Creek	15	15	15	15	15	15
Conococheague	10	10	10	10	10	10
Eastern Bay/Kent Narrows/Lower Chester River/Langford Creek/ Kent Island Bay	10	10	10	10	10	10
Gunpowder River/Lower Gunpowder Falls/Bird River/ Middle River-Browns	9	9	9	9	9	9
Jones Falls	9	9	9	10	9	9
Loch Raven Reservoir	9	9	9	10	9	9
Lower Pocomoke	9	9	9	10	9	9
Middle Chester River	9	9	10	10	10	10
Middle Patuxent River	10	10	10	10	10	10
Nanticoke River	9	9	9	10	9	10
Potomac River Lower Tidal/ Potomac River Middle Tidal	7	7	10	7	7	7
Potomac River Montgomery County	14	14	14	14	14	14
Potomac River Washington County/Marsh Run/Tonoloway/ Little Tonoloway	9	9	11	9	9	9
Rocky Gorge Dam	10	10	10	10	10	10
Savage River	16	16	16	16	16	16
South River/West River	9	9	9	9	9	9
Town Creek	8	8	8	8	8	8
TOTAL	187	187	193	195	188	189
PSU 2003						
Potomac River Lower North Branch	15	15	15	15	15	15
Georges Creek	10	10	10	10	10	10
Antietam Creek	12	12	12	12	12	12
Lower Monocacy	20	20	20	20	20	20
Catoctin Creek	12	12	12	12	12	12
Rock Creek/Cabin John Creek	10	10	10	10	10	10
Liberty Reservoir	15	15	15	15	15	15
St. Mary's River	10	10	10	10	10	10
Magothy/Severn Rivers	9	9	10	10	10	10
Port Tobacco River	10	10	10	10	10	10
West Chesapeake Bay	10	10	10	10	10	10
Little Gunpowder Falls	9	9	9	9	9	9
Broad Creek	10	10	10	10	10	10
Lower Elk River PSU	10	10	10	10	10	10
Miles/Wye Rivers	10	10	10	10	10	10
Middle Chester River	10	10	10	10	10	10
Honga River PSU	10	10	10	10	10	10
Tuckahoe Creek	9	9	9	10	9	9
Pocomoke Sound PSU	10	10	10	10	10	10
TOTAL	211	211	212	213	212	212

Table 6-10. (Continued)	Number of Sites Fished	Number of Summer Habitat Sites	Number of Summer Water Quality Sites	Number of Sites - Amphibians and Reptiles	Number of Sites - Mussels	Number of Sites - SAV
PSU 2004						
Little Youghiogheny/ Deep Creek Lake	10	10	10	10	10	10
Evitts Creek	10	10	10	10	10	10
Wills Creek	9	9	9	9	9	9
Little Conococheague/Licking Creek	10	10	10	10	10	10
Potomac River Frederick County	9	9	9	10	9	9
Anacostia River	15	15	15	15	15	15
Bodkin Creek/Baltimore Harbor	10	10	10	10	10	10
Gwynns Falls	10	10	10	10	10	10
Patuxent River (Lower)	18	18	18	18	18	18
Patuxent River (Upper)	9	9	9	10	9	9
Potomac Lower Tidal/Potomac Middle Tidal	9	9	9	10	9	9
Wicomico River	10	10	10	10	10	10
Lower Susquehanna/Octoraro Creek/Conowingo Dam Susquehanna River	10	10	10	10	10	10
Deer Creek	14	14	14	14	14	14
Lower Winters Run/Atkisson Reservoir	10	10	10	10	10	10
Bush River/Bynum Run	10	10	10	10	10	10
Upper Chester River	10	10	10	10	10	10
Marshyhope Creek	9	9	9	10	9	9
Fishing Bay/Transquaking River	9	9	9	10	9	10
TOTAL	201	201	201	206	201	202

Highlands streams; new benthic IBIs were developed for the Coastal Plain, Eastern Piedmont, and Highlands streams. The addition of one new fish IBI and one new benthic IBI reduced the natural variability found in these assemblages. At the same time, smaller streams (i.e., those draining catchments < 300 ac), which have been sampled more frequently since 2000, were included in the reference conditions used to develop the new IBIs. The resultant new IBIs have good to excellent classification efficiencies (80% to 90%) and are well balanced between Type I and Type II errors. Appropriately higher IBI scores for coldwater streams, smaller streams, and to some extent blackwater streams are significant improvements over the original IBIs. Overall, about 20% fewer watersheds in Maryland are designated as degraded using the new IBIs. The new IBIs remain transparent and understandable, and provide clear thresholds of impairment for both the biointegrity and interim (fishable and swimmable) water quality goals. The consistency between the original and new IBIs allows for joint estimates between rounds, detection of trends in stream condition, and minimal impact on county programs. The new fish and benthic IBIs are shown in Tables 6-11 and 6-12.

6.8.2 Physical Habitat Indicator

Physical stream habitat is the physical template upon which the biological structure of stream communities is built. Degradation of the physical habitat has serious consequences for stream communities and is among the leading cause of stream impairment nationwide (USEPA 2000). Therefore, an important component of the MBSS assessment program is developing a reference-based indicator of physical habitat conditions.

The MBSS has been collecting a variety of physical habitat measures for streams in the state since 1994. In 1999, the MBSS developed a provisional physical habitat index (PHI) to synthesize those extensive data into a single multimetric indicator of physical habitat quality.

Table 6-11. The new fish IBI metrics by region and their threshold values (metrics adjusted for catchment size are indicated by *)			
	Thresholds		
Fish IBIs (metrics)	5	3	1
Coastal Plain			
Abundance sq/m	≥ 0.72	0.45 – 0.71	< 0.45
Number of Benthic species *	≥ 0.22	0.01 – 0.21	0
Percent Tolerant	≤ 68	69 - 97	> 97
Percent Generalist, Omnivores, Insectivores	≤ 92	93 - 99	100
Percent Round-bodied Suckers	≥ 2	1	0
Percent Abundance Dominant Taxa	≤ 40	41 - 69	> 69
Eastern Piedmont			
Abundance sq/m	≥ 1.25	0.25 – 1.24	< 0.25
Number of Benthic species *	≥ 0.26	0.09 – 0.25	< 0.09
Percent Tolerant	≤ 45	46 – 68	> 68
Percent Generalist, Omnivores, Insectivores	≤ 80	81 - 99	100
Biomass sq/m	≥ 8.6	4.0 – 8.5	< 4.0
Percent Lithophilic Spawners	≥ 61	32 - 60	< 32
Warmwater Highlands			
Abundance sq/m	≥ 0.65	0.31 – 0.64	< 0.31
Number of Benthic species *	≥ 0.25	0.11 – 0.24	< 0.11
Percent Tolerant	≤ 39	40 – 80	> 80
Percent Generalist, Omnivores, Insectivores	≤ 61	62 – 96	> 96
Percent Insectivores	≥ 33	1 – 32	< 1
Percent Abundance of Dominant Taxa	≤ 38	39 - 89	> 89
Coldwater Highlands			
Abundance sq/m	≤ 0.88	0.89 – 2.24	> 2.24
Percent Tolerant	≤ 0.22	0.23 – 0.81	> 0.81
Percent Brook Trout	≥ 0.14	0.01 – 0.13	< 0
Percent Sculpins	≥ 0.44	0.01 – 0.43	< 0

The provisional PHI has been used to assess the physical condition in Maryland streams, but several aspects of the index needed refinement. In 2002, the MBSS updated and revisited the provisional PHI (Paul et al. 2002). Additional habitat metrics were investigated for their potential to improve the characterization, especially the extent to which they might help predict biological condition (Table 6-13).

The new PHI provides a valuable physical habitat assessment tool that addresses concerns associated with the provisional PHI. It discriminates between reference and degraded sites and is correlated to biological condition. The new PHI is an improvement over the provisional PHI in that it (1) removed the use of fish IBI scores in the reference criteria and thus the bias toward sites with high fish scores, (2) removed the watershed area effects implicit in many of the habitat measures, (3) removed the trash metric from the PHI which was considered non-habitat, (4) removed embeddedness from the coastal plain sites, which naturally lack coarse sediments, and (5) was better correlated with both fish and benthic biological

Table 6-12. The new benthic macroinvertebrate IBI metrics by region and their threshold values

Benthic IBI*s* (metrics)	Thresholds		
	5	**3**	**1**
Coastal Plain			
Number of Taxa	≥ 22	14 – 21	< 14
Number of EPT Taxa	≥ 5	2 – 4	< 2
Number of Ephemeroptera Taxa	≥ 2	1 - 1	< 1
Percent Intolerant Urban Taxa	≥ 28	10 – 27	< 10
Percent Ephemeroptera Taxa	≥ 11	0.8 – 10.9	< 0.8
Number of Scraper Taxa	≥ 2	1 – 1	< 1
Percent Climbers Taxa	≥ 8	0.9 – 7.9	< 0.9
Piedmont			
Number of Taxa	≥ 25	15 – 24	< 15
Number of EPT Taxa	≥ 11	5 – 10	< 5
Number of Ephemeroptera Taxa	≥ 4	2 – 3	< 2
Percent Intolerant Urban Taxa	≥ 51	12 – 50	< 12
Percent Chironomidae Taxa	≤ 4.6	4.7 – 63	> 63
Percent Clinger Taxa	≥ 74	31 – 73	< 31
Combined Highlands			
Number of Taxa	≥ 24	15 – 23	< 15
Number of EPT Taxa	≥ 14	8 – 13	< 8
Number of Ephemeroptera Taxa	≥ 5	3 – 4	< 3
Percent Intolerant Urban Taxa	≥ 80	38 – 79	< 38
Percent Tanytarsini Taxa	≥ 4	0.1 – 3.9	< 0.1
Percent Scraper Taxa	≥ 13	3 – 12	< 3
Percent Swimmer Taxa	≥ 18	3 – 17	< 3
Percent Diptera Taxa	≤ 26	27-49	> 50

Table 6-13. The new Physical Habitat Index (PHI) metrics by region

PHIs **(metrics)**		
Coastal Plain	**Eastern Piedmont**	**Highlands**
Total Bank Stability	Riffle Quality	Embeddedness
Wood	Total Bank Stability	Total Bank Stability
Instream Habitat	Wood	Epifaunal Substrate
Epifaunal Substrate	Instream Habitat	Total Shade
Total Shade	Epifaunal Substrate	Riparian Width
Remoteness	Total Shade	Remoteness
	Remoteness	

indices. The new PHI has yet to be validated. In addition, the MBSS is considering adding sediment texture and bed stability metrics in the future as both were significantly correlated with biological condition in streams from two Maryland counties where these measures were made – one Piedmont and one Coastal Plain.

6.9 FIELD AND LABORATORY METHODS

6.9.1 Spring and Summer Index Periods

Benthic macroinvertebrate and water quality sampling were conducted in spring, when acidic deposition effects are often the most pronounced. While it is recognized that several different index periods may be used for benthic sampling, the MBSS chose the spring index period because this is the best time to get macroinvertebrates in their most easily identifiable stage of development. Fish, amphibian, reptile, and aquatic vegetation surveys, along with physical habitat evaluations, were conducted during the low-flow period in summer. Fish community composition tends to be stable during summer, and low flow is advantageous for electrofishing.

Because low-flow conditions in summer may be a primary factor limiting the abundance and distribution of fish populations, habitat assessments were performed during the summer and coincided with fish sampling.

To reduce temporal variability, sampling was conducted within specific, relatively narrow time intervals, referred to as index periods. The spring index period was defined by degree-day limits for specific parts of the state. The spring index period was between March 1 and about May 1, with the end of the index period determined by degree-day accumulation as specified in Hilsenhoff (1987). In 2000-2004, most of the spring samples were collected by the end of April, well before degree-day accumulation limits were approached. The targeted summer index period was between June 1 and September 30 (Kazyak 2000). In 2000-2004, most of the summer sampling was completed by the end of September, before the end of the targeted index period. However, in 2004, two sites were sampled in October. While the spring index period is two months in duration because of changing weather conditions (possible rapid warming leading to changes in stream condition), the summer index period is four months long because weather conditions are more consistent throughout the season and fish sampling is more time-consuming.

6.9.2 Water Chemistry

During the spring index period, water samples were collected at each site for analysis of water quality conditions, with an emphasis on factors related to acidic deposition and nutrients (Table 6-14). Grab samples were collected in 0.5 and 1-liter bottles for analysis of all analytes except pH. Water samples for pH were collected with 60 ml syringes, which allowed purging of air bubbles to minimize changes in carbon dioxide content (EPA 1987). Samples were stored on wet ice and shipped on wet ice to the analytical laboratory within 48 hours. The requirement to filter for some analytes within 48 hours was exceeded by several hours for some samples, but never by more than 12 hours. Laboratory analyses were carried out by the University of Maryland's Appalachian Laboratory in Frostburg.

Chemical analysis of water samples followed standard methods as listed in Table 6-14. Routine daily quality control (QC) checks included processing duplicate, blank, and calibration samples according to EPA guidelines for each analyte. Field duplicates were taken at 5% of all sites. Routine QC checks helped to identify and correct errors in sampling routines or instrumentation at the earliest possible stage. Standard operating procedures were implemented that detailed the requirements for the correct performance of analytical procedures. The internal QA/QC protocols followed guidelines outlined in EPA (1987). The complete QA/QC report for 2000-2004 MBSS including laboratory analysis can be found in Roth et al. (2005).

During the summer index period, in situ measurements of dissolved oxygen (DO), pH, temperature, and conductivity were collected at each site to further characterize existing water quality conditions that might influence biological communities. Measurements were made at an undisturbed section of the segment, usually in the middle of the stream channel, and at the upstream segment boundary, using electrode probes. Instruments were calibrated daily and calibration logbooks were maintained to document instrument performance. In 2000-2004, there were no quality assurance problems apparent in log books and other documentation (Rogers et al. 2004).

6.9.3 Benthic Macroinvertebrates

Benthic macroinvertebrates were collected to provide a semi-quantitative description of the community composition at each sampling site. Benthic community data were

Table 6-14. Analytical methods used for water chemistry samples collected during the spring index period

Analyte (units)	Method	Instrument	Detection Limit	Holding Time (days)
pH (standard units)	EPA (1987) Method 19	Orion pH meter	0.01	7
Acid neutralizing capacity (µeq/l)	EPA (1987) Method 5	Brinkmann Automated Titration System equipped with customized software	0.01	14
Sulfate (mg/l)*	EPA (1987) Method 11	Dionex DX-500 Ion Chromatograph (AS-9 HC column)	0.03	14
Nitrite nitrogen* (mg/l)	EPA (1999) Method 354.1	Lachat QuikChem Automated Flow Injection Analysis System	0.0005	28 (frozen)
Nitrate nitrogen* (mg/l)	EPA (1987) Method 11	Dionex DX-500 Ion Chromatograph (AS-9 HC column)	0.01	14
Ammonia (mg/l)*	EPA (1999) Method 350.1	Lachat QuikChem Automated Flow Injection Analysis System	0.003	28 (frozen)
Total nitrogen (mg/l)*	APHA (1998) 4500-N (B)	Lachat QuikChem Automated Flow Injection Analysis System w/In-line Digestion Module	0.050	28 (frozen)
Orthophosphate (mg/l)*	APHA (1998) 4500-P (G)	Lachat QuikChem Automated Flow Injection Analysis System	0.0010	28 (frozen)
Total phosphorus (mg/l)*	APHA (1998) 4500-P (I)	Lachat QuikChem Automated Flow Injection Analysis System w/In-line Digestion Module	0.0013	28 (frozen)
Chloride (mg/l)*	EPA (1987) Method 11	Dionex DX-500 Ion Chromatograph (AS-9 HC column)	0.02	14
Specific conductance (µmho/cm)	EPA (1987) Method 23	YSI Conductance Meter w/Cell	0.1	7
Dissolved organic carbon (mg/l)*	EPA (1987) Method 14	Dohrmann Phoenix 8000 Organic Carbon Analyzer	0.14	28
Particulate carbon (mg/l)	D'Elia et al. (1997)	CE Elantech N/C Analyzer	0.0595	

* Indicates analyses that require filtration within 48 hours

collected primarily for the purpose of calculating DNR's Benthic Index of Biotic Integrity (BIBI) for Maryland streams (Stribling et al. 1998). Recognizing that Maryland streams vary from high-gradient riffle habitat with abundant cobble substrate to low-gradient Coastal Plain streams with sandy or silty bottoms, MBSS employs a "D" net suitable for sampling a wide variety of habitats. This multi-habitat approach is consistent with the recommendations of the Mid-Atlantic Coastal Streams Workgroup (MACS 1996) and the EPA's most recent Rapid Bioassessment Protocols (Barbour et al. 1999).

At each segment, a 600-micron mesh "D" net was used to collect organisms from habitats likely to support the greatest taxonomic diversity. These habitats often include a riffle area when present. Other habitats, in order of preference, included gravel, broken peat, or clay lumps in a run area; snags or logs that create a partial dam or are in run habitat; undercut banks and associated root mats; and SAV and detrital/sand areas in moving water. In riffles and most other habitats, sampling involved placing the net downstream, gently rubbing surficial substrates by hand to dislodge organisms, and disrupting deeper substrates using vigorous foot action. Each dip of the net covered one to two square feet, and a total of approximately 2.0 m^2 (20 square feet) of combined substrates was sampled; samples were preserved in 70% ethanol. Duplicate benthic samples were taken at 15 MBSS sites to assess the replicability of the field methods.

In the laboratory, the preserved sample was transferred to a gridded pan and organisms were picked from randomly selected 1 X 1 inch grid cells until the cell that contained the 100th individual (if possible) was completely picked. Some samples had fewer than 100 individuals. The benthic macroinvertebrates were identified to genus, or lowest practicable taxon, in the laboratory. To aid in identification, oligochaete and chironomid taxa were slide-mounted and identified under a microscope. Laboratory QC procedures included the re-subsampling and identification of every 20th sample. This second sample was identified according to standard procedures and comparisons were made between the two duplicates. For the 2000-2004 sampling year, samples from 75 sites (7%) were re-subsampled for QC purposes. The MBSS voucher specimen collection is currently maintained at the Maryland DNR Field Office in Annapolis, Maryland. A complete description of laboratory protocols can be found in Boward and Friedman (2000) and results of the QC analysis can be found in Roth et al. (2005).

In macroinvertebrate monitoring, the decision to employ a particular subsample size (100 vs. 200 or greater) reflects a balance of how to best utilize program effort. While a larger subsample may improve precision in characterizing individual sites, each sample then requires additional effort for laboratory identification. If a program goal is better precision in characterizing watersheds, the added effort might be spent on sampling more sites per watershed. At the outset of the MBSS monitoring program, a decision was made that 100-organism subsamples would provide acceptable precision at the single site level, and that, within a given total cost, effort would instead be focused on maximizing the total number of sites that could be sampled. However, DNR is interested in further investigating the effect of 100- vs. 200-organism subsampling to improve site assessments and for biodiversity inventory purposes.

6.9.3.1 Stream Waders-Volunteer Benthic Sampling Procedure

Stream benthic macroinvertebrates were sampled by Stream Wader volunteers. MBSS samples were collected at the watershed level (8-digit), while Stream Waders volunteers sampled at the subwatershed (12-digit) level. Thus, Stream Waders data should help "fill in the gaps" left in watershed areas not sampled by MBSS.

Each year, local government agencies and citizen organizations interested in the selected watersheds (the same watersheds chosen to be sampled that year by the core MBSS) are invited to submit site locations to be sampled by Stream Waders volunteers. For 2000-2004 sampling, about 2877 sites were chosen by local government agencies and citizen organizations. These pre-selected sites, along with others chosen to support DNR-supported programs (e.g., Watershed Restoration Action Strategies) were prioritized over others. For subwatersheds with few or no pre-selected sites, volunteers were asked to distribute additional sites throughout the subwatershed, with one site near the most downstream portion of the catchment. Most sites were either upstream of a road crossing or within an easy walk of a road. Volunteers selected 100-foot sections of stream for their samples. Each team of volunteers was given a GPS unit to record the latitude and longitude of the actual sampling sites.

A total of 755 volunteers were trained during three to five eight-hour training sessions in February of 2000-2004. For 2000-2004, 746 12-digit watersheds were slated for sampling. Each of the 211 volunteer teams that formed during the training sessions were asked to select four subwatersheds and to sample five sites within each subwatershed. Volunteers sampled during the same spring index period used by the MBSS (March-April).

Benthic macroinvertebrates were sampled using the same methods as MBSS biologists (Boward 2001 and Kazyak 2000). Samples were preserved in ethanol and organisms were subsampled (about 100 organisms per sample) and identified to family level (Boward and Friedman 2000) by DNR staff at DNR's laboratory in Annapolis. From the list of organisms identified from each site, a family-level Index of Biotic Integrity (IBI) was calculated and each site was rated as Good (IBI 4-5), Fair (IBI 3-3.9), or Poor (IBI 1-2.9)(Stribling et al. 1998).

6.9.4 Fish

Fish were sampled during the summer index period using double-pass electrofishing within 75-m stream segments. Block nets were placed at each end of the segment and direct current backpack electrofishing units were used to sample the entire segment. An attempt was made to thoroughly fish each segment on each pass, sampling all habitats within the entire stream segment. A consistent effort was applied over the two passes. This sampling approach allowed calculation of several metrics constituting the biological index and produced estimates of fish species abundance.

In all streams, at least one anode for every 3-m of stream width was used. Captured fish from each pass were identified to species, weighed in aggregate, counted, and released. Any individuals that could not be identified to species were retained for laboratory confirmation, and a voucher series of about 10 individuals was retained for each major (Maryland 6-digit) drainage basin. For each pass, all individuals of each gamefish species (defined as trout, bass, walleye, northern pike, chain pickerel, and striped bass) were measured for total length. Gamefish species are those with length restrictions for recreational fishing and trout. For each species, unusual occurrences of visible external pathologies or anomalies were noted.

All voucher specimens and fish retained for positive identification in the laboratory were examined and verified by Dr. Rich Raesley, an ichthyologist at Frostburg State University, Frostburg, Maryland. All MBSS collections are archived in the fish museum at Frostburg State University.

6.9.5 Amphibians and Reptiles

At each segment sampled during spring and summer, amphibians and reptiles found during the course of electrofishing and other activities were captured, identified, and recorded. Individuals were identified to species when possible, but larval salamanders and tadpoles were not retained for identification. One initial voucher specimen was retained for each species found for all watersheds. Thereafter, photo documentation was retained for all questionable specimens. A photographic voucher collection is kept by the DNR.

6.9.6 Mussels

During the summer index period, freshwater mussels were sampled by visual inspection at each 75-m stream segment. The presence of Unionid mussels or Asiastic clam (*Corbicula fluminea*) was recorded as live, old shell, or recent shell.

6.9.7 Aquatic and Streamside Vegetation

During the summer index period, aquatic vegetation was sampled qualitatively by examining each 75-m stream segment for the presence of aquatic plants. The presence and relative abundance of submerged, emergent, and floating aquatic vegetation were recorded.

In addition, the presence and relative abundance of invasive terrestrial plant species (e.g., multiflora rose) were recorded during summer sampling.

6.9.8 Physical Habitat

Habitat assessments were conducted during spring and summer sampling at all stream segments as a means of assessing the importance of physical habitat to the biological integrity and fishability of freshwater streams in Maryland. Procedures for habitat assessment (Kazyak 2000) were derived from two commonly used methodologies: EPA's Rapid Bioassessment Protocols (RBPs) (Plafkin et al. 1989), as modified by Barbour and Stribling (1991), and the Ohio EPA's Qualitative Habitat Evaluation Index (QHEI) (Ohio EPA 1987, Rankin 1989).

During spring, riparian zone vegetation type and width on each bank was estimated to the nearest meter (up to 50 meters from stream). Severity and type of buffer breaks were noted. Local land use type and the extent and type of stream channelization were recorded and stream gradient was measured. Crews also recorded distance from road and assigned a trash rating (based on visible signs of human refuse at a site).

During summer sampling, several habitat characteristics (instream habitat, epifaunal substrate, velocity/depth diversity, pool/glide/eddy quality, and riffle/run quality) were assessed qualitatively on a 0-20 scale, based on visual observations within each segment. The percentage of embeddedness of riffles and the percentage of shading of the stream site were visually estimated. Also recorded were the extent and severity of bank erosion and bar formation, number of large woody debris and rootwads within the stream channel, and the presence of various stream features such as substrate types, various morphological characteristics, and beaver ponds. Maximum depth within the segment was measured. Wetted width, thalweg depth, and thalweg velocity were measured at four transects. A complete velocity/depth profile was taken at one transect to compute discharge (streamflow); for sites too shallow to use a flow meter, the speed of a floating object was substituted to allow calculation of discharge.

Recognizing that water temperature is an important factor affecting stream condition (but one that varies daily and seasonally), the MBSS deployed temperature loggers at most sites. In some cases, the same logger was used for two sites if they were close together on the same reach. A single Onset Computer Corporation Optic Stowaway model temperature logger was anchored in each sample site during the summer index period. They recorded the water temperature every 20 minutes from approximately June 1 until September 1. Field crews had the option of retrieving the loggers during summer sampling if the site was visited after August 15. Also, if a site was nearly dry in the spring, field crews may have elected not to deploy a logger.

6.10 QUALITY ASSURANCE

Quality assurance and quality control (QA/QC) are integral parts of the data collection and management activities of the MBSS. The MBSS employs well-established QA/QC procedures, as detailed in Kazyak (2000). Some key points are highlighted below.

6.10.1 Data Management

All crews used standardized pre-printed data forms developed for the MBSS to ensure that all data for each sampling segment were recorded and standard units of measure were used. Using standard data forms facilitates data entry and minimizes transcription error. The field crew leader and a second reviewer checked all data sheets for completeness and legibility before leaving each sampling location. Original data sheets were sent to the Data Management Officer for further review, another signoff, and data entry, while copies were retained by the field crews.

A custom database application (written in Microsoft Access), in which the input module was designed to match each of the field data sheets, was used for data entry. Data were independently entered into two databases and compared using a computer program as a quality-control procedure. Differences between the two databases were resolved from original data sheets or through discussions with field crew leaders.

6.10.2 QA/QC for Field Sampling

A Quality Control Officer (QC Officer) experienced in all aspects of the MBSS was appointed to administer the quality assurance program. Specific quality assurance activities administered by the QC Officer included preparing a field manual of standard sampling protocols, designing standard forms for recording field data, conducting field crew training and proficiency examinations, conducting field and laboratory audits, making independent habitat assessments, identifying taxa, reviewing all reports, and reporting errors.

To ensure consistent implementation of sampling procedures and a high level of technical competency, experienced field biologists were assigned to each crew and all field personnel completed program training before participating in field sampling. Training topics included MBSS program orientation, stream segment location using global positioning system (GPS) equipment, sampling protocols, operation and maintenance of sampling equipment, data transcription, quality assurance/ quality control, and safety. The spring field crews received additional training in sampling protocols for water quality and benthic macroinvertebrates. The summer field crews received additional training in habitat assessment methods, fish, reptile, and amphibian taxonomy, and *in situ* water chemistry assessment.

Training included classroom, laboratory, and field activities. Instructors emphasized the objectives of the MBSS and the importance of strict adherence to the sampling protocols. The QC Officer conducted proficiency examinations to evaluate the effectiveness of the training program and ensure that the participants had detailed knowledge of the sampling protocols. Members of the spring sampling crew were required to demonstrate proficiency in techniques for collecting samples for water chemistry and benthic macroinvertebrates. At least one member of each summer sampling crew was required to pass a comprehensive fish taxonomy examination. Each crew also demonstrated proficiency in locating pre-selected stream segments using the GPS receiver and determining if the segment was acceptable for sampling. Comprehensive "dry runs" were conducted to simulate actual field conditions and evaluate classroom instruction.

Field audits were conducted by the QC Officer during the field sampling to assess the adequacy of training, adherence to sampling protocols, and accuracy of data transcription. The audits included evaluation of the preparation and planning prior to field sampling, stream segment location using GPS equipment and assessment of acceptability for sampling, adherence to sampling protocols, data transcription, and equipment maintenance and calibration. The QC Officer made an independent assessment of habitat at all segments where field audits were done (approximately 11% of the total number of sites).

A separate QA report (Roth et al. 2005) reports on details of QA activities for the 2000-2004 sampling years.

6.10.2.1 Stream Waders QA

The Stream Waders Program contained several measures of QA/QC including testing of laboratory taxonomists and repeated subsampling and sample re-identification (MDNR 2004). Volunteers attended a one-day training session and were asked to repeat the training each year that they participated in the program. Three percent of Stream Waders sites were sampled by DNR staff (duplicate samples) during the March-April index period (a gap of three or more weeks occurred between collection of the volunteer samples and the quality control samples due to the time needed for the volunteers to complete sampling and return the samples to DNR). Duplicate samples were also subsampled and identified by DNR staff according to MBSS protocols.

Site locations were verified by comparing the latitudes and longitudes recorded by volunteers using the GPS units with other location information from the data sheets such as stream name, nearest road crossing, and map grid.

6.11 REFERENCES

American Public Health Association (APHA). 1998. Standard Methods for the Examination of Water and Wastewater, 20th Edition. American Public Health Association, Washington, D.C.

Angermeier, P.L. and M.R. Winston. 1999. Characterizing fish community diversity across Virginia landscapes: prerequisite for conservation. Ecological Applications 9:335-349.

Barbour, M.T., and J.B. Stribling. 1991. Use of habitat assessment in evaluating the biological integrity of stream communities. *In*: Biological Criteria: Research and Regulation. U.S. Environmental Protection Agency, Washington, D.C. EPA-440/5-91-005. pp. 25-38.

Barbour, M.T., J. Gerritsen, B.D. Snyder, and J.B. Stribling. 1999. Rapid Bioassessment Protocols for Use in Streams and Wadeable Rivers: Periphyton, Benthic Macroinvertebrates and Fish, Second Edition. U.S. Environmental Protection Agency, Office of Water, Washington, D.C. EPA 841-B-99-002.

Boward, D. 2001. Maryland Stream Waders Volunteer Stream Monitoring Manual. Maryland Department of Natural Resources, Monitoring and Non-Tidal Assessment Division.

Boward, D. and E. Friedman. 2000. Maryland Biological Stream Survey Laboratory Methods for Benthic Macroinvertebrate Laboratory Processing and Taxonomy. Maryland Department of Natural Resources, Monitoring and Non-tidal Assessment Division. CBWP-MANTA-EA-00-6.

Chesapeake Coastal Watershed Service, 2000. Lower Eastern Shore Conservation and Restoration Action Strategy. Maryland Department of Natural Resources.

Cochran, W.G. 1977. Sampling Techniques. 3rd ed. New York: John Wiley and Sons.

Collett, D. 1999. Modeling Binary Data. Chapman & Hall/CRC. 369 pp.

Clean Water Action Plan Technical Workgroup. 1998. Maryland Clean Water Action Plan: Final 1998 Report on Unified Watershed Assessment, Watershed Prioritization and Plans for Restoration Action Strategies. www.dnr.state.md.us/cwap/cwap.htm.

D'Elia, C.F., E.E. Connor, N.L. Kaumeyer, C.W. Keefe, K.V. Wood, C.F. Zimmerman. 1997. Nutrient Analytical Services Laboratory Standard Operating Procedures, Technical Report Series No. 158-97. Chesapeake Biological Laboratory, University of Maryland Center for Environmental Science.

Efron, B. 1982. The jackknife, the bootstrap and other resampling plans. Regional conference series in applied mathematics. The Society for Industrial and Applied Mathematics, Philadelphia, PA.

Environmental Protection Agency (EPA). 1987. Handbook of Methods for Acid Deposition Studies: Laboratory Analyses for Surface Water Chemistry. Office of Acid Deposition, Environmental Monitoring and Quality Assurance, U.S. Environmental Protection Agency, Washington, DC.

Environmental Protection Agency (EPA). 1999. Methods and Guidance for Analysis of Water. EPA 821-C-99-004. Office of Water, U.S. Environmental Protection Agency, Washington, D.C.

ESRI (Environmental Systems Research Institute, Inc.). 1998. ArcView, version 3.X: Redlands, California.

Gauch, H.G., Jr. 1982. Multivariate analysis in community ecology. Cambridge Studies in Ecology. Cambridge University Press. Cambridge, England.

Hawkes, C.L., D.L. Miller, and W.G. Layher. 1986. Fish ecoregions of Kansas: stream fish assemblage patterns and associated environmental correlates. Environmental Biology of Fishes 17:267-279.

Heimbuch, D.G., H.T. Wilson, S.B. Weisberg, J.H. Vølstad, and P.F. Kazyak. 1997. Estimating fish abundance in stream surveys by using double-pass removal sampling. Transactions of the American Fisheries Society 126:795-803.

Hilsenhoff, W.L. 1987. An improved biotic index for organic stream pollution. Great Lakes Entomologist 20:31-39.

Horvitz, D.G. and D.J. Thompson. 1952. A generalization of sampling without replacement from a finite universe. *Journal of the American Statistical Association* 47: 663-685.

Hughes, R.M., E. Rexstad, and C.E. Bond. 1987. The relationship of aquatic ecoregions, river basins, and physiographic provinces to the ichthyogeographic regions of Oregon. Copeia 1987:423-432.

Jessen, R.J. 1978. Statistical Survey Techniques. John Wiley, New York.

Johnson, Zoë P. 2000. A Sea Level Rise Response Strategy for the State of Maryland. Maryland Department of Natural Resources, Coastal Zone Management Division.

Kazyak, P.F. 1994. Maryland Biological Stream Survey Sampling Manual. Prepared by Versar, Inc., for Maryland Department of Natural Resources, Chesapeake Bay Research and Monitoring Division.

Kazyak, P.F. 2000. Maryland Biological Stream Survey Sampling Manual. Maryland Department of Natural Resources, Monitoring and Non-tidal Assessment Division.

Korn, E.L. and B.I. Graubard. 1999. Analysis of Health Surveys. John Wiley & Sons. New York.

Lyons, J. 1989. Correspondence between the distribution of fish assemblages in Wisconsin streams and Omernik's ecoregions. American Midland Naturalist 122:163-182.

Maryland Department of Planning, Maryland State Data Center. 2004. Projections by Type for All Counties - Historic Census 1970 to 2000, Projected 2005 to 2030, http://www.mdp.state.md.us/msdc/dw_popproj.htm.

Maryland DNR Fisheries Service. 2001. Fish Blockages GIS coverage.

Matthews, W.J. and H.W. Robison. 1988. The distribution of the fishes of Arkansas: a multivariate analysis. Copeia 1988:358-374.

McCune, B., and M.J. Medford. 1997. PC-ORD: Multivariate analysis of ecological data, version 3.0. MJM Software Design, Gleneden Beach, Oregon.

MDNR. 2004. Maryland Department of Natural Resources. Maryland Stream Waders Sample Year 2003 Report. CBWP-MANTA-EA-04-2, Annapolis, MD. 26 pp. http://www.dnr.state.md.us/streams/pubs/ea04-2stw.pdf.

Mid-Atlantic Coastal Stream Workgroup (MACS). 1996. Standard Operating Procedures and Technical Basis: Macroinvertebrate collection and habitat assessment for low-gradient nontidal streams. Delaware Department of Natural Resources and Environmental Conservation, Dover, DE.

National Atmospheric Deposition Program (NRSP-3). 2005. NADP Program Office, Illinois State Water Survey, 2204 Griffith Dr., Champaign, IL 61820.

Omernik J.M. and R.G. Bailey. 1997. Distinguishing between watersheds and ecoregions. Journal of the American Water Resources Association 33(5):935-949.

Plafkin, J.L., M.T. Barbour, K.D. Porter, S.K. Gross and R.M. Hughes. 1989. Rapid bioassessment protocols for use in streams and rivers: Benthic macroinertebrates and fish. U.S. Environmental Protection Agency, Office of Water, Washington, D.C. EPA 440-4-89-001.

Rankin, E.T. 1989. The Qualitative Habitat Evaluation Index (QHEI): Rationale, methods, and application. Ohio EPA, Division of Water Quality Planning and Assessment, Ecological Analysis Section, Columbus, OH.

Rao, J.N.K. 2003. Small Area Estimation. John Wiley and Sons. 313 pp.

Redistricting Census. 2000. TIGER/Line Files Technical Documentation. Prepared by the U.S. Census Bureau-Washington, D.C.

Rogers, G., J. Vølstad, N. Roth, and M. Southerland. 2004. Maryland Biological Stream Survey 2003 Quality Assurance Report. Prepared by Versar, Inc., for Maryland Department of Natural Resources, Monitoring and Non-Tidal Assessment Division.

Roth, N., G. Rogers, L. Erb, and J. Vølstad. 2005 Maryland Biological Stream Survey Quality Assurance Report. 2005. Prepared by Versar, Inc., for the Maryland Department of Natural Resources, Monitoring and Non-tidal Assessment Division.

Roth, N.E., M.T. Southerland, G. Mercurio, J.C. Chaillou, P.F. Kazyak, S.S. Stranko, A.P. Prochaska, D.G. Heimbuch, and J.C. Seibel. 1999. State of the Streams: 1995-1997 Maryland Biological Stream Survey Results. Prepared by Versar, Inc., Columbia, MD, and Post, Buckley, Schuh, and Jernigan, Inc., Bowie, MD, with Maryland Department of Natural Resources, Monitoring and Non-Tidal Assessment Division. CBWP-MANTA-EA-99-6.

Roth, N.E., M.T. Southerland, J.C. Chaillou, H.T. Wilson, D.G. Heimbuch, J.C. Seibel. 1998. Maryland Biological Stream Survey: Ecological Assessment of Non-Tidal Streams Sampled in 1996. Prepared by Versar, Inc., and Post, Buckley, Schuh and Jernigan, Inc., for Maryland Department of Natural Resources, Monitoring and Non-Tidal Assessment Division. CBWP-MANTA-EA-98-1. NTIS No. PB99-122913.

SAS Institute Inc. 1999. SAS OnlineDoc®, Version 8, Cary, NC: SAS Institute Inc.

Schenker, N. and J.F. Gentleman. 2001. On judging the significant of differences by examining the overlap between confidence intervals. The American Statistician 55(3):182-186.

Smith, John. 1999. Maryland Surface Water Balance Database. Maryland Department of the Environment, Baltimore, MD.

Southerland, M., G. Rogers, P. Kazyak, M. Kline, D. Boward, R. Morgan, and S. Stranko. 2005. Development of New Fish and Benthic Macroinvertebrate Indices of Biotic Integrity for Maryland Streams. Unpublished manuscript.

Southerland, M., N. Roth, G. Mercurio, and J. Vølstad. 2000. Final Design and Procedures for MBSS 2001-2004 (Round Two). Memorandum to R. Klauda and P. Kazyak, Maryland Department of Natural Resources, Monitoring and Non-Tidal Assessment Division.

Strahler, A.N. 1957. Quantitative analysis of watershed geomorphology. Transactions of the American Geophysical Union 38:913-920.

Strahler, A.N. 1957. Quantitative analysis of watershed geomorphology. Transactions of the American Geophysical Union 38(6):913-920.

Stribling J.B., B.K. Jessup, J.S. White, D. Boward, and M. Hurd. 1998. Development of a Benthic Index of Biotic Integrity for Maryland Streams. Prepared by Tetra Tech, Inc., and Maryland Department of Natural Resources, Monitoring and Non-Tdal Assessment Program. CBWP-MANTA-EA-98-3.

Thompson, S K. 1992. Sampling. John Wiley & Sons. New York. 343 pp.

Titus, James G. 1998. Rising Seas, Coastal Erosion, and the Takings Clause: How to Save Wetlands and Beaches Without Hurting Property Owners. Maryland Law Review 57(4):1307.

Vølstad, J.H., N.K. Neerchal, N.E. Roth, and M.T. Southerland. 2002. Combining biotic indices of stream condition from multiple surveys in a Maryland Watershed, Biological Indicators.

Vølstad, J.H., M.T. Southerland, S.B. Weisberg, H.T. Wilson, D.G. Heimbuch, and J.C. Seibel. 1996. Maryland Biological Stream Survey: the 1994 Demonstration Project. Prepared by Versar, Inc., for Maryland Department of Natural Resources, Monitoring and Non-Tidal Assessment Division. CBWP-MANTA-EA-95-9.